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QUANTITY OF MILK OBTAINED FROM AMPUTATED COW UDDERS [1]

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INTRODUCTION

For several years the Bureau of Dairy Industry, United States Department of Agriculture, has been carrying on research work in milk secretion for the purpose of learning not only when and how milk is secreted, but to what extent abundance of secretion is associated with the history of the udder's growth and development, and with its external and internal characteristics. The results obtained by hand milking the amputated udders of two cows slaughtered at the regular hour of milking, were reported in a previous publication (*9*).[3] The milk was drawn in two separate milkings, one as soon as possible after the amputation of the udder, the other four hours later. One of these udders yielded 85 per cent and the other 49 per cent of the average quantity obtained at corresponding milkings before death. The smaller proportion of milk obtained from the second amputated udder was attributed to rigor mortis and excessive excitement and struggling of the cow at the time of death. The results reported offered convincing evidence that milk secretion is to a great extent a continuous process, that a large proportion of the milk obtained at a milking is stored within the udder before milking is commenced, and that the capacity of the secretory system of a cow's udder is greater than the volume of milk obtained at a milking. These deductions were distinctly contradictory to the belief, which for a long time had persisted rather generally among teachers and other professional men in dairy cattle and veterinary work, that the capacity for storing milk in the cow's udder is not more than a half pint to each quarter, and that consequently nearly all of the milk obtained at any milking must have been secreted during the process of milking. The manipulation of the udder and teats was supposed to stimulate the rapid secretion of milk during this brief period.

DISCUSSION OF RECENT WORK OF OTHER INVESTIGATORS

Since the report covering the results obtained with the first two cows was submitted for publication, several investigators have reported results on work of a similar nature. Gaines and Sanmann (*1*), by

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[3] Reference is made by number (italic) to Literature Cited, p. 400.

chemical analysis of amputated udders and their contents, from cows slaughtered at the regular milking time, recovered an amount of lactose equal to that obtained in the milk from the same udders at corresponding milkings before death. Three amputated udders were included in their study. One of the udders and its contents was analyzed as a unit. The other two udders were milked by hand, and the milk so obtained was analyzed separately from the udder with its residual contents. The lactose found in the three amputated udders was, respectively, 100, 96, and 118 per cent of the quantity found by analysis in the corresponding ante-mortem milkings. These results led them (*1, p. 701*) to infer that—

> * * * there is present in the udder at milking time an amount of milk in excess of the amount secured upon milking. Very roughly, one-fourth of the milk is obtainable by milking the amputated udder, another fourth by drainage from the sliced udder, while the remaining half is tenaciously held even in the finely ground tissues.

Gowen and Tobey (*2, 3*) conducted a similar experiment based on a determination of the quantity of lactose in the udder and its contents at the time of the death of the cow. They concluded that at the time of milking 80 to 85 per cent of the milk is present in the udder of a cow milking up to 30 pounds a day, and that the amount which may be secreted during milking is about 10 to 15 per cent, but can not be more than 20 per cent of the yield of the cow. Another interesting result noted is the failure to recover significant quantities of milk by subjecting the udder tissue to a pressure of as much as 2,000 pounds. In 16 out of 20 cases, the quantity of lactose found in the amputated udder was more than sufficient to account for the total lactose produced by the cow at a corresponding ante-mortem milking. The average for the 20 udders showed an excess of lactose equivalent to 2.1 pounds of the average milk produced by these cows. This was assumed to represent the quantity of milk remaining in the udder after it was believed to have been milked dry. They conclude (*3, p. 127*) that cows producing up to 30 pounds of milk at one milking "have the lactose equivalent of all this milk in the udder when milking commences." The conclusions of Gowen and Tobey are greatly strengthened by data which show that the nonlactating udder does not contain any lactose.

Ragsdale and his associates (*7*) obtained 69 per cent of the previous yield of milk from an udder of one cow after she had been killed. In a second experiment the amputation of the udder was followed by six post-mortem milkings at half-hour intervals. The total quantity of milk recovered by post-mortem milking was 11.3 pounds as compared with an average of 10 pounds for the nine days before death. A later report (*8*) gave results obtained with two additional cows; the quantities of milk recovered from the two amputated udders were, respectively, 36.5 and 57.96 per cent of the quantities obtained at corresponding ante-mortem milkings.

Petersen (*5, p. 143*) reports results obtained by milking six amputated udders, and concludes:

> 1. That the udder of the cow can and does contain all of the milk drawn at a milking. 2. In all probability the milk is secreted at a more or less uniform rate in the interim between milkings. 3. That post-mortem milks are normal in all respects except their fat content, which is low.

In a later report Petersen, Palmer, and Eckles (*6*) describe their work somewhat more fully. For five of the six cows, the right half of the udder was milked immediately before slaughter and the left half was milked post mortem.

According to the writers' conception, "milk secretion" is the process carried on by the secretory cells of the udder in removing certain constituents from the blood and converting them into milk. Milk secretion is distinguished from the act of "giving down" or expelling the secreted product. The act of "giving down" the milk varies greatly with different cows and probably may be stimulated and accelerated by the manipulation of the udder during milking. If the manipulation stimulates release or expulsion of milk already secreted, in all probability it would affect the half of an udder not milked before slaughter at least as much as the half milked immediately before slaughter. This being so, it should result in more ready removal and a relatively greater post-mortem recovery than otherwise. The results for the five cows handled in this manner by Petersen and his associates show that the average recovery of milk from the left half of the amputated udder exceeded by 5 per cent the quantity obtained from the right half immediately before slaughter. However, a comparison of the average quantity of milk obtained post mortem with that obtained from the same half of the udder at corresponding ante-mortem milkings shows a relative post-mortem recovery of only 77.8 per cent when the five cows are considered, and 80.3 per cent when the six cows are included.

The low production of the cows studied by Petersen and his associates is noted also. The average quantity of milk obtained from the left half of the six udders at corresponding ante-mortem milkings was 1,981 g, or 4.37 pounds. The average quantity obtained from the same half of the same six udders after death was 1,590.3 g, or 3.51 pounds. Almost identical production of the two halves of the udder in ante-mortem milkings of the six cows is shown. This would indicate for both halves of the six udders an average ante-mortem production of 8.74 pounds and an average post-mortem production of 7.02 pounds.

Olson and Copeland [4] refer to unpublished data which in some respects are in accord and in other respects in contrast to those obtained elsewhere. Four cows of varying production were studied in a somewhat similar manner. Each of these cows was milked twice daily while the ante-mortem producing level was being determined. Cow No. 1 was producing 46.67 pounds of milk daily before slaughter and yielded on two post-mortem milkings 13.1 and 1.3 pounds, a total of 14.4 pounds, which was about 62 per cent of the ante-mortem production at a corresponding milking. Cow 2 was producing an average of 6.88 pounds daily. Nearly 24 hours after the last ante-mortem milking she was killed, and 2.61 pounds was drawn from one half of her udder. Cow 3 was yielding an average of 8.85 pounds daily. Nearly 24 hours after the last ante-mortem milking she was slaughtered. Just before death 3.72 pounds of milk had been removed from one half of her udder. Immediately after death the other half of the udder yielded 2.51 pounds. The writers' comment relative to a

[4] Olson, T. M., and Copeland, L. Unpublished data on file at the South Dakota Agricultural Experiment Station.

similar plan employed by Petersen and his associates applies to the results obtained with this cow. Cow 4 was yielding an average of 20.61 pounds daily. By continuous massaging and milking of the udder for 1½ hours after death 8.75 pounds was obtained. Because of the lack of uniformity in the handling of these cows, a measure of the relation between the quantities of milk obtained by ante-mortem and post-mortem milking is difficult. It appears, however, that the post-mortem recovery for the four cows was 61.70, 75.87, 56.72, and 84.87 per cent, respectively, and averaged approximately 70 per cent.

DESCRIPTION OF EXPERIMENTS AND RESULTS OBTAINED WITH FOUR COWS

The methods used and the results obtained in studying the first two cows (Nos. 459 and 292) were fully described in a previous report (*9*). After the preparation of that report and before its publication two other cows (Nos. 123 and 272) were studied similarly. (Table 1.)

TABLE 1.—*Quantity of milk obtained before death of the cow and after death and amputation of the udder*

[Cows in Group 1 felled by a blow on the head and udders permitted to become chilled after death; cows in Group 2 shot and udders held at blood temperature]

Group and cow No.	Average production for 10 days, ante-mortem milking	Milk from first post-mortem milking	Milk from second post-mortem milking	Total post-mortem milk	Percentage of ante-mortem production obtained in post-mortem milkings
Group 1:	*Pounds*	*Pounds*	*Pounds*	*Pounds*	
459	[a] 12.07	9.20	1.07	10.27	85.09
292	21.38	7.70	2.90	10.60	49.58
123	10.37	5.90	1.30	7.20	69.43
272	21.83	6.50	2.30	8.80	40.31
Average	16.41	7.33	1.89	9.22	61.10
Group 2:					
846	18.51	15.40	3.30	18.70	101.03
903	16.27	9.00	2.60	11.60	71.30
811	15.20	8.90	.80	9.70	63.82
257	24.73	12.10	3.25	15.35	62.07
253	21.25	12.55	2.90	15.45	72.71
443	17.41	11.85	3.35	15.20	87.31
255	25.80	16.15	1.65	17.80	68.99
Average	19.88	12.28	2.55	14.83	75.32
Average for Groups 1 and 2	18.62	10.48	2.31	12.79	70.15

[a] 3-day average.

Cow 123, a junior 4-year-old registered Guernsey weighing 810 pounds, was slaughtered at 9.30 a. m. on December 2, 1926. She had produced 8,535 pounds of milk and 477 pounds of fat in one year at the age of 2 years, 5 months. She was in the seventh month of her second lactation period. Her udder was unbalanced, the right half being smaller than the left, and the right rear quarter being entirely inactive. For a number of days before death she was led each morning from the barn to the slaughterhouse and back so that the trip on the day of slaughter would not cause undue excitement. Every attempt was made to acquaint her with her new surroundings, but

her udder was not handled nor was she in any way treated to stimulate the letting down of milk either on previous days or on the morning of slaughter. For the 10 days previous to slaughter her production ranged from 9.8 to 11 pounds and averaged 10.37 pounds when she was milked once daily. Her schedule of feeding and watering was kept as nearly uniform as possible. She was killed by a blow on the head and subsequent bleeding. Death was reasonably sudden and without excessive struggle. The temperature of the air in the slaughterhouse was close to freezing on the day of slaughter. Forty minutes elapsed between time of killing and commencement of milking, during which time the udder was amputated and attached to an iron frame.

The time required for daily milking before death was 5 to 7 minutes. The post-mortem milk was drawn more slowly and with somewhat greater difficulty, the first milking requiring 34 minutes, and the second, 10 minutes. The temperature of the milk during the 10 days before death ranged from 34° to 36° C. The temperature of the milk of the first post-mortem milking was approximately 29½° near the beginning and 25° near the finish. The temperature of the milk of the second and final post-mortem milking was approximately 25½°. The temperature of the inside of the udder was not determined. The udder remained in the slaughterhouse for nearly two hours, after which it was removed to a room having a temperature of about 24° to 27°, where it was kept until final milking was complete. Undoubtedly the udder was chilled considerably during the first two hours after its amputation.

Cow 272, a senior 4-year-old registered Holstein weighing 1,200 pounds, was slaughtered at 9.30 a. m. on December 22, 1926. Her best record of production for one year, which was made at the age of 2 years, amounted to 12,762 pounds of milk and 447 pounds of fat. She was in the seventh month of her third lactation period when killed. Her udder was large, well attached, slightly tilted, and slightly quartered, but functioning in all quarters. On December 18 the left front quarter showed an infection and a reduced milk flow. The infected quarter responded to treatment, and on December 20 and 21 appeared again to be normal in condition and in quantity of production. For 10 days before death she was led from the barn to the slaughterhouse and back each day, as in the case of cow 123. During this 10-day period, her production ranged from 20.0 to 24.2 pounds and averaged 21.83 pounds on one milking daily. Her schedule of feeding and watering was kept as nearly constant as possible. She was killed by a blow on the head and subsequent bleeding. Several strokes were required to fell her. She struggled and kicked vigorously, and her muscles apparently were tense at and immediately after death. Considerable importance is attached to the possible effects of failure to obtain sudden death without excessive struggling. The temperature of the air in the slaughterhouse was low. Forty-five minutes elapsed between killing and commencement of milking, during which time the udder was amputated and suspended.

The time required for daily milking before death was 7 to 11 minutes. At each milking on December 18 to 21, inclusive, the cow was milked as before, for 9 to 10 minutes, and the major portion of the milk so obtained was weighed. The udder was then massaged and stripped for 15 minutes more. The massaging and stripping resulted in the recovery of an additional 0.1 to 0.5 of a pound of milk

daily, and for the four days averaged 0.25 of a pound. The post-mortem milking was much slower and more difficult. In the first 9 minutes only 4 pounds of milk had been drawn, and its temperature was approximately 29½° C. At the end of 45 minutes 6.5 pounds of milk had been drawn, and its temperature was about 22°. The second post-mortem milking occupied 25 minutes. The temperature of this milk also was 22°. The temperature of the milk drawn during the 10 days before death had ranged from 36° to 37°. The temperature of the inside of the udder was not determined. The udder remained in the slaughterhouse at a low temperature for about two hours, after which it was sent to a warm room, where it remained until after the second post-mortem milking. Undoubtedly this udder, like the previous one, was decidedly chilled before it was removed to the warm room.

On an average, the first four amputated udders yielded 61.10 per cent as much milk as the cows had produced at corresponding milkings before death. (Table 1.) Those yielding the greatest quantity before death liberated a lower percentage of that quantity after death. The udders of the two cows that struggled violently at the time of slaughter yielded only 49.58 and 40.31 per cent, respectively, whereas the udders of the two cows that died without excessive struggle or noticeable tension of muscles liberated 85.09 and 69.43 per cent, respectively, of the quantities produced at similar milkings before death.

The smaller proportion of milk liberated after death, and the greater difficulty in drawing it from the amputated udders of the two cows that became excited, struggled violently, and died with tense muscles, suggest the possibility that these factors may have been responsible for the difficulty experienced in drawing the milk as readily or as completely as from the others. In this connection, it is suggested that the nervous shock produced by killing the cow and to some extent by severing the nerves in the process of amputating the udder, may result in the development of an abnormal condition in the udder more or less comparable to that produced by pain or fright, which apparently causes the living cow to hold up her milk.

CONTINUATION OF EXPERIMENTS UNDER MORE CAREFULLY CONTROLLED CONDITIONS WITH SEVEN COWS

The necessity for repeating the experiments under more carefully controlled conditions was apparent. Therefore, in the second group of experiments, the results of which are presented in Tables 1 and 2, an attempt was made (1) to prevent as far as possible all excitement of the cow before death, (2) to cause sudden death without excessive struggling, and (3) to maintain approximately blood temperature within the amputated udder until after the completion of post-mortem milking.

As a beginning for this study, three grade Holstein cows were obtained from the dairy herd of St. Elizabeths Hospital in Washington, D. C. They were in average flesh and condition, but were inclined to be high-strung, nervous, and excitable. The cows were placed in box stalls in the building where slaughter was to take place, and were kept as quiet as possible. A regular program of feeding, watering, and milking was adopted. In an effort to obtain sudden death without

excessive strugging, the cows were shot through the brain. In order that the inside of the udder might be held approximately at blood temperature, a wooden box was constructed of sufficient size to hold the iron frame from which the udders were suspended. (Fig. 1.) The box was provided with an electric heater, a glass window in the front for purposes of visibility, and a slide in the end to serve as a ventilator. Below the front window an opening was provided and

FIGURE 1.—Box for controlling temperature of the udder in post-mortem milking

covered with a heavy canvas. A thermometer was suspended within the box in such a position that readings could be made from the outside.

Immediately after the cow was shot cloths soaked in hot water were wrapped about the udder. Frequently changed hot cloths were kept on the udder during the time required for suspending and bleeding the cow and amputating the udder, attaching it to the frame, and inserting it in the box. From the time the udder was inserted until the final post-mortem milking was completed, the temperature of the

box was kept at about 47° to 48° C. A thermometer, inserted in one of the large blood vessels, permitted a determination of the inside temperature of the gland. Milking was commenced at once. It was accomplished by reaching through slits in the canvas front and milking in the usual manner. The udder was visible to the milker through the window.

Cow 846, a 6-year-old grade Holstein weighing 1,075 pounds, was slaughtered at 9.30 a. m. on April 6, 1927. Her best record for one year was 12,220 pounds of milk made at the age of 4 years 9 months, on two milkings daily. At the time of slaughter she was beginning the fifth month of lactation. Her udder apparently was normal, relatively small, and somewhat tilted but unusually loose, flexible, and mellow, with comparatively little tissue present when milked out. During the 10 days immediately before slaughter her production ranged from 17.4 to 19.7 pounds and averaged 18.51 pounds when she was milked once daily. Shooting through the brain resulted in almost instantaneous death with hardly any struggle or apparent tenseness of muscles. She was bled immediately. Forty-eight minutes was required for killing and bleeding her and for amputating the udder, attaching it to its supporting frame, and placing it in the temperature-control box for milking.

The quantity of milk obtained was 15.4 pounds in the first and 3.3 pounds in the second post-mortem milking, a total of 18.7 pounds or 101.03 per cent of the average daily production for the 10 previous days. Milking of the amputated udder was remarkably easy and rapid. The major portion of the first post-mortem milking was drawn in 6 minutes. Massaging and stripping was continued for 7 minutes. The second post-mortem milking occupied 11 minutes. During the four days immediately before death the time required for milking the major portion varied from 6 to 7 minutes. Subsequent massaging and stripping on these days yielded only 0.3 to 0.4 of a pound daily. The temperature of the milk during the four days before death ranged from 34° to 36° C. The temperature of the major portion of the first post-mortem milking was 35°, whereas at the end of the stripping period it was 33°. The temperature of the second post-mortem milking was 35½° C. Some difficulty was experienced in reading these temperatures because of the position of the milk bucket in the temperature-control box and because of the milk foam which adhered to the thermometer. The temperature of the control box was maintained between 45° and 49° until after completion of the second post-mortem milking. The temperature of the inside of the udder registered between 37° and 38°. The air temperature at the slaughterhouse was 18½° at 2.30 p. m.

Cow 903, a 5-year-old grade Holstein weighing 1,050 pounds, was slaughtered at 9.30 a. m. on April 11, 1927. Her best production for one year was 12,943 pounds of milk, made at the age of 3 years 11 months, on two milkings daily. She was in her fifteenth month of lactation. Her udder was of medium size, fair shape, except for being light in the left rear quarter, very loose, yielding, and mellow, but inferior in quality to that of cow 846. Her milk production for the previous 10 days ranged from 15.3 pounds to 17.7 pounds and averaged 16.27 pounds when she was milked once daily. Death by shooting was practically instantaneous and without struggle. She

was bled immediately. Forty-five minutes elapsed between the time of shooting the cow and commencement of post-mortem milking.

The quantity of milk obtained by hand milking was 9 pounds in the first and 2.6 pounds in the second post-mortem milking, a total of 11.6 pounds, which is 71.3 per cent of the average quantity obtained during the previous 10 days. Post-mortem milking was comparatively easy and rapid, but less so than in the case of cow 846. The major portion of the first post-mortem milking was drawn in 10 minutes, after which the udder was massaged and milked for 9 minutes. The second post-mortem milking occupied 11 minutes. During the nine days before death the time required for drawing the major portion had ranged from 6 to 10 minutes. Subsequent massaging and stripping on these days had yielded only 0.4 to 0.8 of a pound daily. The temperatures of the milk during the nine days before death were 34° and 35° C. The temperature was 35° for the major part and 33° for the strippings of the first, and 34½° for the second post-mortem milking. The temperature in the control box was maintained at 45½° to 48° throughout the period of post-mortem milkings. The temperatures of the inside of the udder during the same period ranged from 36½° to 39°. The outside air temperature was 12° at 9.30 a. m. and 14½° at 2.30 p. m.

Cow 811, a 6-year-old grade Holstein weighing 1,280 pounds, was slaughtered at 9.15 a. m. on April 15, 1927. Her best production for one year was 10,928 pounds of milk, made at the age of 4 years 9 months, on two milkings daily. She was just commencing her twenty-fifth month of lactation. Her udder was medium to small, of fair shape, with light and almost dry left rear quarter and with garget in the left front quarter. The udder was loose and yielding, and contained fibrous tissue of medium softness. In quality it was the poorest of the three in the group. Her milk production during the 10 days previous to slaughter ranged from 11.7 to 17.6 pounds and averaged 15.2 pounds on one milking daily. She was shot through the brain, and death was practically instantaneous and without struggle. Bleeding and amputation of the udder followed in rapid succession. Forty-four minutes elapsed between shooting and commencement of milking.

The quantity of milk obtained by hand milking the amputated udder was 8.9 pounds for the first and 0.8 pound for the second post-mortem milking, a total of 9.7 pounds or 63.82 per cent of the average quantity yielded during the previous 10 days. The milking was fairly easy and rapid but not so readily accomplished as in the case of cow 846. Seven minutes was required for drawing the major portion, and 6 minutes for massaging the udder and drawing the remainder of the first post-mortem milk. The second post-mortem milking required 10 minutes. During the previous 10 days the time required for drawing the major portion had ranged from 6 to 10 minutes and the temperature of the milk had ranged from 34° to 36° C. On these days subsequent massaging and stripping had yielded 0.2 to 0.5 of a pound. The temperature of the milk was 37° for the major portion and 34½° for the strippings of the first post-mortem, and 33° for the second post-mortem milking. In this case a third post-mortem milking was attempted approximately two hours after the second, for the purpose of determining whether

additional milk could be obtained in significant quantities. Six minutes of massaging and stripping yielded only approximately 0.05 of a pound. The temperature of the control box was maintained between 45° and 50° until the completion of the third post-mortem milking except that immediately after the udder was inserted, the temperature reading was 38°. The temperature of the inside of the udder during this period ranged from 37° to 39°. Outside air temperatures on this day were 12½° at 9.30 a. m. and 17° at 2 p. m.

From time to time lactating cows in the herd of the Bureau of Dairy Industry at Beltsville, Md., became available for similar experiments. The work performed with each of four additional cows, making a total of seven in the second group, is discussed separately.

Cow 257, a 10-year-old registered Holstein, was slaughtered at 9.15 a. m. on February 21, 1928. She weighed 1,194 pounds, which was considerably below her usual mature weight. Her best production record for one year was 24,135 pounds of milk and 852 pounds of fat, made at 6 years 4 months of age on three milkings daily. She was in the second month of lactation when slaughtered. Her udder was somewhat unbalanced, of good size, fair shape, and very loose, and contained tissue that was inclined to be hard and fibrous. She had been kept on a regular schedule of feeding and watering and was milked twice daily throughout a 10-day period. The morning milking commenced at 9.15 a. m. Production at the morning milking during this 10-day preliminary period ranged from 21.6 to 27.7 pounds and averaged 24.73 pounds. Death, which resulted from shooting through the brain, was almost instantaneous and was accompanied with very little struggling or tenseness of muscles. Bleeding of the animal and amputation of the udder followed in rapid succession. Sixty-three minutes elapsed between shooting and commencement of milking.

Hand milking of the amputated udder yielded 12.10 pounds in the first and 3.25 pounds in the second post-mortem milking, a total of 15.35 pounds or 62.07 per cent of the average quantity obtained during the previous 10 days. In order to make possible a subsequent study, the two halves of the udder were milked separately in both the first and second post-mortem milkings. The time required for drawing the milk, therefore, was somewhat greater than otherwise would have been necessary. Ten minutes was required for drawing the major portion in the first post-mortem milking, after which the udder was massaged and stripped. The second post-mortem milking required 17 minutes. The time for drawing the major portion of the milk at corresponding milkings during the preliminary period, when both halves were milked simultaneously, ranged from 8 to 12 minutes and averaged 10 minutes, and the temperature of the milk, which was taken on the last six days, ranged from 35° to 38° C. During the 10-day period, subsequent massaging and stripping yielded from 0.5 to 1 pound and averaged 0.8 of a pound of milk. The temperature was 32½° for the major portion and 22° and 26½° for the two separate parts of the strippings of the first post-mortem milking. The temperature of the second post-mortem milk was 27½° for each part.

The temperature within the control box ranged from 45° to 52° C., and the temperature of the inside of the udder ranged from 36° to 38°. The air temperature in the slaughter room was approximately

1½° at the time of slaughter and 4½° at the end of the second post-mortem milking.

Cow 253, a 9-year-old registered Holstein weighing 1,263 pounds, was slaughtered at 9.19 a. m. on June 11, 1928. Her best production record for one year was 16,405 pounds of milk and 628 pounds of fat, made at 5 years 8 months of age on three milkings daily. She had been lactating 14 months at the time of slaughter. Her udder was of medium to good size, medium shape, and excellent flexibility, but the tissue within was medium to hard and very fibrous. She had been fed and watered regularly and milked once daily at 9.15 a. m. for 10 days, during which her production ranged from 19.3 to 22.5 pounds and averaged 21.25 pounds daily. The first shot from a 22-caliber rifle was not effective and the cow became violent. A second shot was effective, but her muscles became extremely tense. Bleeding and amputation of the udder followed as quickly as possible, but 64 minutes elapsed between the time of shooting and the commencement of milking of the amputated udder.

Hand milking yielded 12.55 pounds in the first and 2.90 pounds in the second post-mortem milking, a total of 15.45 pounds or 72.71 per cent of the average quantity obtained at corresponding milkings during the previous 10 days. Eight minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for 7 minutes. The second post-mortem milking required 8 minutes. During the previous 10 days the drawing of the major portion had required 8 to 9 minutes and averaged 8½ minutes, and the temperature of the milk ranged from 37° to 38° C. Subsequent massaging and stripping during the 10-day period yielded an average of 0.71 of a pound. The temperature of the milk of the first post-mortem milking was 34° for the major portion and 29½° at the end of stripping; that of the second post-mortem milking was 32°.

The temperature of the control box ranged from 45° to 52° C. The temperature of the inside of the udder was determined after the first, and again after the second post-mortem milking, and in each case was 38°. The air temperature was 17½° at the time of slaughter and 17° at the completion of the second post-mortem milking.

Cow 443, a 9-year-old registered Jersey weighing 855 pounds, was slaughtered at 9.30 a. m. on June 12, 1928. Her best record of production for one year was 9,621 pounds of milk and 465 pounds of fat, made at 6 years 4 months of age on three milkings daily. She was in the seventh month of lactation when slaughtered. Her udder was large, of good shape, and very loose and yielding, but contained fibrous tissue of more than average mellowness. She had been kept on a regular schedule of feeding and watering and had been milked regularly once daily at 9.30 a. m. through a 10-day period, during which her milk production ranged from 14.6 to 19.5 pounds and averaged 17.41 pounds daily. Death from shooting was almost instantaneous and was accompanied by little struggling, although the muscles became relatively tense. Bleeding and amputation of the udder followed in rapid succession and within 59 minutes from the time of shooting, the udder was placed in the temperature control box and milking was commenced.

Hand milking of the amputated udder yielded 11.85 pounds in the first and 3.35 pounds in the second post-mortem milking, a total of 15.20 pounds or 87.31 per cent of the average quantity obtained in corresponding milkings during the previous 10 days. Twelve minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for four minutes. The second post-mortem milking required eight minutes. During the previous 10 days, the drawing of the major portion had required from 7 to 9 minutes and averaged 7.9 minutes, and the temperature of the milk ranged from 37° to 38° C. Subsequent massaging and stripping on these 10 days yielded an average of 0.72 of a pound. The temperature of the milk obtained at the first post-mortem milking was 34½° for the major portion and 31° at the end of stripping. The temperature of the milk obtained at the second post-mortem milking was 32°. The temperature of the control box ranged from 40° to 60°. This wide variation was due to breakage of the heating coil. The temperature of the udder tissue after the second post-mortem milking was 37°. The air temperature was 19½° at the time of slaughter and 22° at the time of the second post-mortem milking.

Cow 255, a 9-year-old registered Holstein weighing 1,462 pounds, was slaughtered at 9.05 a. m. on February 1, 1929. Her best record of production for one year was 11,202 pounds of milk and 415 pounds of butterfat, made at 2 years 8 months of age on three milkings daily. She was slaughtered 32 days after the last freshening. She had been kept on a regular schedule of feeding and watering, and had been milked once daily at 9 a. m. for 10 days, during which her milk production ranged from 23.1 to 29.2 pounds and averaged 25.8 pounds daily. Her udder was of medium size, poor shape, very loose and mellow, and contained a medium quantity of mellow, fibrous tissue which was inclined to be stringy. Death from shooting was almost instantaneous and was accompanied by a minimum of struggling or tenseness of muscles. Bleeding and amputation of the udder followed, and 79 minutes after the cow was shot, milking in the temperature control box was commenced.

Hand milking yielded 16.15 pounds in the first and 1.65 pounds in the second post-mortem milking, a total of 17.80 pounds or 68.99 per cent of the average quantity obtained at corresponding milkings during the previous 10 days. The milk was drawn easily and rapidly. Eleven and one-half minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for an equal period of time. Massaging and stripping yielded only 1.1 pounds of milk. The second post-mortem milking required 11 minutes. During the previous 10 days the drawing of the major portion of the milk had required from 9 to 11 minutes and averaged 10.2 minutes, and the temperature of the milk on the last 8 of these days had ranged from 34° to 36° C. Subsequent massaging and stripping during this 10-day period yielded an average of 1.05 pounds. The temperature of the milk obtained at the first post-mortem milking was 33° for the major portion and 31½° at the end of stripping; that of the second post-mortem milk was 30°. The temperature of the control box ranged from 22½° when the udder was inserted to 50° at the time of final milking. The low temperature at the start resulted from an attempt to generate sufficient heat with

a small electric plate when the air temperature was −1° in the slaughter room. Soon after the udder was inserted a different heating unit was used and the results were more satisfactory. The inside temperature of the udder at the end of the first post-mortem milking was 34½°. This temperature increased steadily, reaching 38½° at the time of the second post-mortem milking. The air temperature in the slaughter room was 3° at 3.30 p. m.

COMPARISON OF RESULTS OBTAINED WITH THE TWO GROUPS OF COWS

In the first group of cows studied an average of 61.10 per cent as much milk was obtained from the amputated udders as from the same udders at corresponding milkings before death of the cows. The minimum post-mortem recovery for cows of this group was 40.31 per cent, and the maximum was 85.09 per cent. In the second group the average post-mortem recovery was 75.32 per cent,with a minimum of 62.07 and a maximum of 101.03 per cent. The average for both groups was 70.15 per cent. These values are obtained by dividing the sum of the percentages in a group by the number of cows in the group. Such a method is permissible, as the work with each cow is considered as an independent experiment having equal importance with every other one.

The average producing level for each cow was 3.47 pounds greater in the second group than in the first, and the quantity of milk obtained from the amputated udders was 5.61 pounds greater in the second group than in the first. If it were true that the milk in the udder at the beginning of milking represents only a small proportion of the quantity obtained at that milking, one would expect a greater percentage of recovery from those amputated udders that had yielded the smaller quantities before death. Such, however, was not the case, as the amputated udders of the second or higher-producing group of cows yielded not only a greater quantity but a greater proportion, as shown by the average post-mortem recoveries of 61.10 and 75.32 per cent, respectively, for groups 1 and 2. Of the total quantity of milk recovered from the amputated udders, 81.94 per cent was obtained at the first post-mortem milking and only 18.06 per cent at the second.

The percentages in Table 1 are, with one exception, based on the average production of each cow at corresponding milkings on the 10 days immediately preceding slaughter. In general the production of milk during the preliminary 10-day period before slaughter was reasonably uniform, but in a few instances production was increasing, which tends to make the percentage showing post-mortem recovery higher than it would have been if only the last two days before slaughter had been used as a basis for comparison. In other instances the cow's production was decreasing, and this resulted in the percentage showing post-mortem recovery being somewhat smaller than it would have been if the comparisons had been made on a 2-day basis.

The post-mortem recovery has been computed also on the basis of the yield for the two days immediately preceding slaughter. (Table 2.) The results are essentially the same whether the percentage of post-mortem recovery of milk from amputated udders is based on the milking level for the 10 days or for the two days preceding slaughter.

TABLE 2.—*Relative post-mortem recoveries of milk from amputated udders, calculated on the basis of the production for the previous 10-day and 2-day periods*

Group and number of cows	Percentage of average quantity obtained on previous 10-day period	Percentage of average quantity obtained on previous 2-day period
Group 1, 4 cows	61.10	61.10
Group 2, 7 cows	75.32	75.52
Groups 1 and 2 combined, 11 cows	70.15	70.27

DISCUSSION

The average recovery of milk obtained by milking the 11 amputated udders (70.15 per cent) was a much greater proportion of the ante-mortem yield than that indicated by the experiments of Gaines and Sanmann.(*1*)

In contrast to the method of milking one half of the udder before and the other half after slaughter, which was employed by Petersen and his associates, (*6*) the writers carefully avoided touching the udder or in any other way stimulating the giving down of the milk immediately before slaughter, except in the case of one of the first cows whose udder was examined for quality of tissue.

Inasmuch as the changes in the manner of killing the cow and in the system of controlling the temperature of the udder after death were both introduced at the same time, the specific effect of each on the quantity of milk recovered from the amputated udders could not definitely be determined. Because of the brevity of the period between killing the cow and commencing the first post-mortem milking, however, it would appear that controlling the temperatures could have had little opportunity to affect the quantity of milk recovered at that milking, and that the greater recovery from amputated udders in Group 2 as compared with that in Group 1 was due chiefly to the effect of changing the method of slaughter. It seems reasonable to assume, however, that during the 4-hour interval between the first and second post-mortem milkings the effect of temperature might have been considerably greater than that of the method of killing on the total quantity of milk obtained.

The high average recovery of milk from amputated udders (61.10 per cent for Group 1, 75.32 per cent for Group 2, and 70.15 per cent for Groups 1 and 2 combined), together with indications that in some cases milk remained in the udders after the second post-mortem milking, which, considering the shock and abnormal conditions incidental to slaughter, is not surprising, offer almost conclusive evidence that practically all the milk obtained at a milking is present in the udder when milking is commenced. Reference to the possibility that the shock of slaughtering may have been partly responsible for the residue of milk observed in some of the udders after completion of the second post-mortem milking suggests the possibility of some mechanism which enables a cow to hold up her milk. This ability of some cows to withhold their milk under certain circumstances is well known. The nature of such a mechanism is not known, but many theories have

been advanced, of which the one expressed by Klein (*4, p. 8–9*) is particularly interesting. From the beginning of the study of this subject the writers' observations have indicated that milk secretion is a continuous process, except when it is interrupted or retarded by such factors as sickness, injury, or pressure in the udder resulting from accumulation of milk. This latter fact also explains why heavy-producing cows yield more milk when milked three or four times daily then when milked only twice daily. The removal of the milk at shorter intervals prevents the development of sufficient pressure to check secretion and permits it to proceed at a uniform rate. It also is in accord with the common practice of drying off cows by allowing the milk to accumulate in the udder. In the drying-off process the pressure developed is evidently sufficient to stop secretion completely until the secreting cells cease to function.

Since the milk is already stored in the udder when milking is commenced, the importance of an adequate size and capacity of udder is obvious.

It has been more or less generally assumed that if a residue of milk is left in the udder at milking time the production of the cow will be diminished if not completely arrested. The necessity for completeness of milking may be questioned, for even though the cow is thoroughly milked at milking time the udder will again contain milk in a very short time.

The fact that a number of investigators using different methods have reported data which lead to almost the same conclusions regarding the continuous secretion and storage of milk in the udder greatly strengthens the deductions outlined in a previous report (*9*), as well as those here presented.

SUMMARY

This paper discusses the individual performance of 11 cows arranged in two groups. The cows in Group 1 were felled by a blow on the head and their udders were permitted to become chilled after death. The cows in Group 2 were killed by shooting and their udders were held at approximately blood temperature until after the second post-mortem milking was completed.

The average recovery of milk from the amputated udders was 61.10 per cent of the ante-mortem yield for Group 1, 75.32 per cent for Group 2, and 70.15 per cent for Groups 1 and 2 combined.

Approximately 80 per cent of the total amount of milk recovered from the amputated udders was obtained at the first and approximately 20 per cent at the second post-mortem milking. Exact figures showing the relation between the quantities obtained at the first and second post-mortem milkings are 79.50 per cent and 20.50 per cent for Group 1, 82.81 per cent and 17.19 per cent for Group 2, and 81.94 per cent and 18.06 per cent for Groups 1 and 2 combined. The relation between the recoveries of milk in the first and second post-mortem milkings is, therefore, almost the same in Group 2 as in Group 1.

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COMPOSITION OF MILK OBTAINED FROM AMPUTATED COW UDDERS[1]

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INTRODUCTION

In an early report (*7*)[3] of the milk secretion studies which have been in progress for several years in the Bureau of Dairy Industry, United States Department of Agriculture, the quantity and composition of the milk obtained from two amputated cow udders was compared with that obtained from the same udders at corresponding milkings before the death of the cow. Another paper (*8*), dealing with quantity of milk, gave a similar comparison of the results obtained with 11 cow udders and showed that most, if not all, of the milk obtained at a milking is present in the udder when milking is commenced. The results given also indicated that milk secretion is a continuous process except when it is interrupted or slowed down by such factors as sickness, injury, or pressure in the udder resulting from the accumulation of milk.

The milk obtained from the two amputated udders (*7*), as compared with that obtained at the last two corresponding ante-mortem milkings, differed more in butterfat than in any other constituent. In the milk drawn at the first post-mortem milking, which took place as soon as possible after amputation of the udder, and in the milk of the second post-mortem milking, performed four hours later, the butterfat test averaged approximately 61 and 26 per cent, respectively, as high as it did in the milk obtained at the last two corresponding ante-mortem milkings. This reduction in butterfat in the milk obtained after death was attributed to the fact that the amputated udders were allowed to become chilled and that, as a result, some of the butterfat may have solidified and adhered to the lining of the ducts.

The percentage of total solids in the milk obtained from the first two cows (*7*) showed a steady decline from the ante-mortem to the first post-mortem and the second post-mortem milkings. The percentage of solids not fat was slightly higher in the milk from the first post-mortem milking than in the ante-mortem milk, but it was lower in the milk from the second post-mortem milking than in either the ante-mortem or the first post-mortem milk. The percentage of ash increased steadily from the ante-mortem to the second post-mortem milking. The percentage of total protein was less consistent but showed a very slight upward trend from the ante-mortem to the first post-mortem milking, and a somewhat greater downward trend from the first to the second post-mortem milking, with the second post-mortem slightly lower in percentage of total protein than the ante-

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[3] Reference is made by number (italic) to Literature Cited, p. 418.

mortem milking. The percentage of lactose showed a moderate upward trend from ante-mortem to first post-mortem and a sharp decline from first to second post-mortem milking.

DISCUSSION OF WORK OF OTHER INVESTIGATORS

Gaines and Sanmann (*1*) concluded that at the time of milking there is present in the udder of the cow a quantity of milk in excess of that obtained upon milking. This conclusion was based on the fact that they were able to recover a greater quantity of lactose from the hashed, amputated udder and its contents than from the milk obtained from the same udders at corresponding ante-mortem milkings. Similarly, Gowen and Tobey (*2*) were able to recover from 16 of 20 udders studied, more than enough lactose to account for the quantity of milk obtained at corresponding milkings before death. The conclusions of Gowen and Tobey appear to be essentially the same as those of Gaines and Sanmann. The conclusions drawn by both of these groups of investigators, however, seem to be based on the assumption that the lactose content of milk is stable and that the recovery of lactose from the amputated udder indicates the presence of milk having the same lactose content. In a later publication Gowen and Tobey (*3*) showed that, at the time of milking, there are also sufficient quantities of fat, ash, and nitrogen to more than account for all the milk produced. Their earlier deductions are greatly strengthened by evidence that the nonlactating udder does not contain any lactose.

Ragsdale and his associates (*6*) working with two amputated udders, obtained 36.50 per cent and 57.96 per cent of the quantity of milk obtained at corresponding ante-mortem milkings, but only 15.90 per cent and 46.44 per cent, respectively, of the normal amount of butterfat. This would indicate that in the first case the butterfat test of the post-mortem milk was only 43 per cent as high, and in the second case only 80 per cent as high as that of the ante-mortem milk; an average for the two of slightly more than 60 per cent.

Petersen (*4, p. 143*) reported that "post-mortem milks are normal in all respects except their fat content, which is low." Petersen, Palmer, and Eckles (*5, p. 579*) reported that "in every case the fat percentage and total yield of fat in the post-mortem milkings are noticeably smaller than in the in-vivo milkings." They also made the interesting observation that when the post-mortem milking was sampled as the milking progressed, the fat percentage rose to a maximum and then dropped almost to the level of the first portion. The weighted average tests for the six cows included in their study were 4.83 per cent for the ante-mortem milking of the left gland, and 3.12 per cent for the post-mortem milking of the left gland. The average test of the post-mortem milk from the left half of the six udders was, therefore, 64 per cent as high as the test of the ante-mortem milk from the left half of the same udders. Their post-mortem recovery of 51.8 per cent as much total butterfat in the left half of the six udders as in the left half of the same udders at corresponding ante-mortem milkings, and their 80.3 per cent recovery of milk on the same basis, also indicate a test 64 per cent as high in the post-mortem as in the ante-mortem milk. It is interesting that the relation of the post-mortem to ante-mortem test observed by Petersen, Palmer, and

Eckles is almost the same as that reported by Ragsdale and his associates.

In contrast to all of the results discussed, are those obtained by Olson and Copeland.[4] Their average butterfat tests of ante-mortem milk, first post-mortem milk, and second post-mortem milk or strippings were 3.73, 3.55, and 6.48 per cent, respectively. In their work the first post-mortem milk tested nearly as high in butterfat as did the ante-mortem milk, and the second post-mortem milk tested nearly twice as high as the first post-mortem milk.

CONDUCT OF EXPERIMENT

As shown in a previous paper (*8*), the cows used in these experiments were put on a uniform schedule of feeding, watering, and milking, which, except in one case, was continued for 10 days. During this preliminary period the lactating level of each cow was determined. The composition of the ante-mortem milk was determined from samples taken on the two days immediately preceding slaughter.

As previously reported (*8*), the 11 cows used in these tests were divided into two groups. Group 1 includes the first four cows studied. These were were killed by a blow on the head, and they became more or less violent at the time of death. Their udders were allowed to remain in the abattoir and become chilled before the post-mortem milking was completed. Group 2 includes seven cows which were studied after the program of control had been better standardized. Each of the seven was killed by shooting, and in nearly every instance death was almost instantaneous. The udders of the seven cows in Group 2 were kept in a control box at approximately blood temperature until after the post-mortem milking was finished.

The quantity of milk obtained in post-mortem milkings of the amputated udders of 11 cows was shown in Table 1 of a previous paper (*8*) to average 70.15 per cent of the quantity obtained at corresponding milkings before death. The average recovery for the first four cows was 61.1 per cent, but the average recovery for the next seven cows, kept and slaughtered under more carefully controlled conditions, was 75.32 per cent of the ante-mortem yield. The report explains that the cows were slaughtered at the exact hour of the day on which the ante-mortem milking had taken place, and that the milk was drawn in 2 separate milkings, 1 as soon as possible after the amputation of the udder, and 1 four hours later.

Samples of the milk obtained at milkings 48 and 24 hours before slaughter, as well as of the milk obtained at both post-mortem milkings, were taken for analysis. The percentages of total solids, ash, and total protein were determined by chemical analysis; the percentage of butterfat was determined by the Babcock method; and the percentages of solids not fat and of lactose were calculated from the analyses obtained.

The average composition of the milk obtained during the last two days before slaughter was used as a basis for comparing the composition of the milk from the first and second milkings of the amputated udders. The quantity of milk obtained on the last two days before slaughter varied only slightly for each cow. Therefore, the average percentage of any constituent in the two ante-mortem samples from

[4] OLSON, T. M., and COPELAND, L. Unpublished data on file at the South Dakota Agricultural Experiment Station.

any cow can be determined with sufficient accuracy by adding the percentages for the two samples and dividing the total by two. The averages which appear in column 2 of Tables 1 to 5, inclusive and Table 7, were obtained in this manner. The relative percentages given for each cow in the fifth and sixth columns of these tables were obtained by dividing the figures in columns 3 and 4, respectively, by the corresponding figures in column 2. The average percentages for Group 1, Group 2, and Groups 1 and 2 combined were obtained by adding the percentages for all cows in the group and dividing each total by the number of cows in the group.

COMPOSITION OF MILK OBTAINED FROM AMPUTATED UDDERS AS COMPARED WITH THAT OBTAINED AT CORRESPONDING ANTE-MORTEM MILKINGS

TOTAL SOLIDS

Table 1 gives the percentages of total solids in the milk obtained before slaughter and in that obtained from the amputated udders. In the results for every cow in Group 1, the percentage of total solids in the milk obtained at the first post-mortem milking was lower than that in the ante-mortem milk, and was still lower in the second post-mortem milk than in the first post-mortem milk. With one exception, the results for the cows in Group 2 showed a similar tendency, although the decline from the first to the second post-mortem milking was considerably less marked. The exception noted was so noticeably out of line with the other results that it was undoubtedly an error. The data for cow 253 were, therefore, omitted from the averages for Group 2 and for Groups 1 and 2 combined. Immediately beneath these averages are given the corresponding ones which include all the data for this cow. The percentage of total solids in the milk was in every instance distinctly lower for the first post-mortem milk than for the ante-mortem milk, and with one exception was still lower for the second post-mortem than for the first post-mortem milk.

TABLE 1.—*Percentages of total solids in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	14.68	13.88	10.23	94.55	69.69
292	10.83	9.61	7.88	88.73	72.76
123	14.23	12.37	10.20	86.93	71.68
272	12.86	10.85	9.19	84.37	71.46
Average	13.15	11.68	9.38	88.65	71.40
Group 2:					
846	13.26	11.69	11.00	88.16	82.96
903	13.25	10.74	10.12	81.06	76.38
811	12.38	10.43	9.73	84.25	78.59
257	10.69	8.71	7.79	81.48	72.87
253	12.14	10.52	[a] 14.35	86.66	[a] 118.20
443	13.17	11.25	11.23	85.42	85.27
255	10.83	9.46	9.37	87.35	86.52
Average	[b] 12.26	10.38	9.87	84.62	80.43
	[c] 12.25	10.40	10.51	84.91	85.83
Average for Groups 1 and 2	[b] 12.62	10.90	9.67	86.23	76.82
	[c] 12.57	10.86	10.10	86.27	80.58

[a] Apparently an error. [b] Data for cow 253 excluded. [c] Data for cow 253 included.

BUTTERFAT

The butterfat percentages are presented in Table 2. The milk from every cow in Group 1 showed a lower percentage of butterfat in the first post-mortem milk, and a still lower percentage in the second post-mortem milk, than in the ante-mortem milk. The butterfat percentages for the milk obtained at the first and second post-mortem milkings averaged only 57.29 and 27.45 per cent as high, respectively, as that of the ante-mortem milkings. The milk from the second post-mortem milking averaged only 47.9 per cent as high in percentage of butterfat as the milk obtained at the first post-mortem milking. In other words, the percentage of butterfat was reduced almost one-half in the first, and again almost one-half in the second, post-mortem milk. The reduction from ante-mortem to second post-mortem milk was fairly uniform for the four cows in the group. The fact that no effort was made to maintain body temperature within the amputated udders strengthens the assumption that a lowering of the udder temperature might have been to some extent responsible for the decrease in percentage of butterfat in the milk obtained from the amputated udders, particularly the decrease from the first to the second post-mortem milking.

TABLE 2.—*Percentages of butterfat in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	5.74	4.50	1.60	78.40	27.87
292	2.93	1.28	.70	43.69	23.89
123	5.43	3.20	1.80	58.93	33.15
272	3.74	1.80	.93	48.13	24.87
Average	4.46	2.70	1.26	57.29	27.45
Group 2:					
846	4.08	2.70	2.93	66.18	71.81
903	4.08	1.93	2.20	47.30	53.92
811	3.70	1.80	1.73	48.65	46.76
257	2.83	1.24	1.33	43.82	47.00
253	3.64	2.10	1.70	57.69	46.70
443	4.24	2.00	2.83	47.17	66.75
255	3.34	1.73	2.60	51.80	77.84
Average	3.70	1.93	2.19	51.80	58.68
Average for Groups 1 and 2	3.98	2.21	1.85	53.80	47.32

The amputated udders of the cows in Group 2 were all kept in the temperature-control box until after the second post-mortem milking was finished. The results for this group differ considerably from those for Group 1. In every instance the percentage of butterfat in the first post-mortem milk was lower than that in the ante-mortem milk, averaging 51.80 per cent as high as that in the ante-mortem milk. To this extent the results were similar to those for Group 1, as the percentage of butterfat was reduced almost one-half. The relation between the butterfat percentages in the first and second post-mortem milks for Group 2, however, was very different from

that shown for Group 1. The milk from five of the seven cows in Group 2 showed a higher percentage of butterfat in the second post-mortem than in the first post-mortem milking. For the other two cows the reverse was true. Averages for Group 2 show that the butterfat test[5] was 51.80 per cent as high in the first, and 58.68 per cent as high in the second post-mortem milk as in the ante-mortem milk. In other words, the decrease in the percentage of butterfat between the ante-mortem milk and the first post-mortem milk obtained from the cows in Group 2 was similar to the corresponding decrease shown for the cows in Group 1, but the second post-mortem milk from the cows in Group 2 showed a higher percentage of butterfat than the first post-mortem milk from the cows in that group.

As previously stated, the chilling of the amputated udders of the cows in Group 1 appears to have been partly, if not entirely, responsible for the reduction in the butterfat test between the first and second post-mortem milkings, since this reduction did not occur when the udders were kept warm. The tendency for a reduction in the butterfat test between the ante-mortem and the first post-mortem milk, however, was not overcome or materially influenced by controlling the temperature of the udder after its amputation.

Since the decreases in percentage of butterfat in the post-mortem milk obtained from udders in Group 2 were distinctly different from those obtained in Group 1, and since these differences in the two series can be accounted for by differences in the temperature at which the udders were held after amputation, the combined averages for Groups 1 and 2 in Table 3 may not be particularly significant. The results for Group 2, however, were obtained under controlled conditions, are reasonably consistent, and should be significant. These results, together with the combined results in both groups, indicate that the butterfat test of milk obtained from amputated udders is only about half as high as that of the milk obtained from the same udders before death.

SOLIDS NOT FAT

In general, the percentages of solids not fat in the milk obtained from cows in Group 1 were higher in the first post-mortem milk than in the ante-mortem milk. (Table 3.) This was true in three of the four cases studied. In each of the four the percentage of solids not fat in the milk from the second post-mortem milking was lower than in the milk of either the ante-mortem or the first post-mortem milking. On an average, the percentages of solids not fat in the milk obtained at the first and second post-mortem milkings were 103.45 and 93.36 per cent, respectively, of the percentages found in the ante-mortem milk.

[5] "Butterfat test" and "percentage of butterfat" are used interchangeably.

TABLE 3.—*Percentages of solids not fat in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	8.94	9.38	8.63	104.92	96.53
292	7.90	8.33	7.18	105.44	90.89
123	8.80	9.17	8.40	104.20	95.45
272	9.12	9.05	8.26	99.23	90.57
Average	8.69	8.98	8.12	103.45	93.36
Group 2:					
846	9.18	8.99	8.07	97.93	87.91
903	9.17	8.81	7.92	96.07	86.37
811	8.68	8.63	8.00	99.42	92.17
257	7.86	7.47	6.46	95.04	82.19
253	8.50	8.42	[c] 12.65	99.06	[c] 148.82
443	8.93	9.25	8.40	103.58	94.06
255	7.49	7.73	6.77	103.20	90.39
Average	[b] 8.55	8.48	7.60	99.21	88.85
	[c] 8.54	8.47	8.32	99.19	97.42
Average for Groups 1 and 2	[b] 8.61	8.68	7.81	100.90	90.65
	[c] 8.60	8.66	8.25	100.74	95.94

[a] Apparently an error. [b] Data for cow 253 excluded. [c] Data for cow 253 included.

For Group 2, however, the results were slightly different. The first post-mortem milk averaged almost the same as the ante-mortem milk in percentage of solids not fat. For five of the cows the percentages of solids not fat were slightly lower and for two they were slightly higher in the first post-mortem than in the ante-mortem milk. The percentages of solids not fat in this group also were decidedly lower in the second post-mortem than in either the ante-mortem or the first post-mortem milk. This was true in six of the seven cases. The only exception in this group was in the case of cow 253, for which the discrepancy in total solids already has been noted. Since the percentages of solids not fat were determined by substracting the percentages of butterfat from the percentages of total solids, any outstanding discrepancy in the latter would be correspondingly great in the percentages of solids not fat. The averages for solids not fat have, therefore, been treated the same as in the case of the total solids. (Table 1.) The averages which include the data for cow 253 are given in Table 3 immediately beneath the other averages.

The downward trends in percentage of solids not fat from the first post-mortem to the second post-mortem milk in Groups 1 and 2 are similar. On an average, the actual percentage of solids not fat in the second post-morten milk was 0.86 per cent lower than that of the first post-mortem milk for Group 1, and 0.88 per cent lower for Group 2. The average figures showing the relation between the solids not fat in the post-mortem and ante-mortem milk are both lower for Group 2 than for Group 1. In general, the first post-mortem milk had almost the same percentage of solids not fat as the ante-mortem milk, but the second post-mortem milk contained only about 90 per cent as high a percentage as the ante-mortem milk. Controlling the temperature of the udder did not prevent a reduction between the first and second post-mortem of about 10 per cent in percentage of solids not fat.

ASH

The percentage of ash showed a definite and consistent upward trend through the post-mortem milkings. (Table 4.) In every instance in both groups, the percentage of ash was higher in the first post-mortem than in the ante-mortem milk, and was still higher in the second than in the first post-mortem milk.

TABLE 4.—*Percentages of ash in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	0.83	0.88	0.94	106.02	113.25
292	.75	.78	.82	104.00	109.33
123	.74	.80	.89	108.11	120.27
272	.76	.79	.85	103.95	111.84
Average	.77	.81	.88	105.52	113.67
Group 2:					
846	.78	.79	.83	101.28	106.41
903	.77	.78	.85	101.30	110.39
811	.74	.79	.86	103.76	116.22
257	.74	.78	.82	105.41	110.81
253	.77	.82	.86	103.59	111.69
443	.78	.81	.87	103.85	111.54
255	.74	.80	.87	108.11	117.57
Average	.76	.80	.85	104.74	112.09
Average for Groups 1 and 2	.76	.80	.86	105.03	112.67

TOTAL PROTEIN

For every individual in Group 1 the percentage of total protein in the first post-mortem milk was as great as that in the ante-mortem milk, or slightly greater. (Table 5.) In every instance, however, it was smaller in the second post-mortem milk than in the first.

TABLE 5.—*Percentages of total protein in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	3.61	3.79	3.63	104.99	100.55
292	2.35	2.35	2.09	100.00	88.94
123	3.21	3.22	3.19	100.31	99.38
272	3.31	3.39	3.22	102.42	97.28
Average	3.12	3.19	3.03	101.93	96.54
Group 2:					
846	4.03	4.00	3.59	99.26	89.08
903	4.69	4.51	4.10	96.16	87.42
811					
257	2.63	2.60	2.47	98.86	93.92
253	3.37	3.60		106.82	
443	3.32	3.39	3.19	102.11	96.08
255	2.60	2.72	2.66	104.62	102.31
Average	[a] 3.45	3.44	3.20	100.20	93.76
	[b] 3.44	3.47	3.20	101.31	93.76
Average for Groups 1 and 2	[a] 3.31	3.33	3.13	100.97	95.00
	[b] 3.31	3.36	3.13	101.56	95.00

[a] Data for cow 253 excluded.

[b] Data for cow 253 included.

In Group 2 the total protein content was not determined for any of the samples from cow 811 or for the second post-mortem milk from cow 253. The average percentages showing the relation of total protein in post-mortem and ante-mortem milk, when based on complete data for the five cows in Group 2, are almost the same as for those in Group 1. The percentage of total protein in the first post-mortem milk appeared to be approximately normal, whereas that of the second post-mortem milk was slightly below normal. This relationship was not materially affected by controlling the temperature of the amputated udders.

As previously stated, the butterfat test showed a downward trend from the ante-mortem to the first post-mortem milking. From the first post-mortem to the second post-mortem milking the trend was sharply downward for Group 1, upward for Group 2, and slightly downward when the averages for Groups 1 and 2 were combined. The percentage of total protein was relatively uniform in the three milkings. Under these circumstances the fat-protein ratio, which is determined by dividing the percentage of butterfat by that of the total protein, would of necessity follow a trend similar to that of butterfat. The fat-protein ratio, which usually is fairly stable in cows' milk, has been determined for the ante-mortem and for the first and second post-mortem milk from each cow, by dividing the percentage of butterfat by that of the total protein. The results are given in Table 6. The milk from every cow in the first group showed a decline in the fat-protein ratio from the ante-mortem to the first post-mortem milking, and an additional decline from the first post-mortem to the second post-mortem milking.

TABLE 6.—*Fat-protein ratios for milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milk	First post-mortem milk	Second post-mortem milk
Group 1:			
459	1.590	1.187	0.441
292	1.247	.545	.335
123	1.692	.994	.564
272	1.130	.531	.289
Average	1.415	.814	.407
Group 2:			
846	1.012	.675	.816
903	.870	.428	.537
811			
257	1.076	.477	.538
253	1.080	.583	
443	1.277	.590	.887
255	1.285	.636	.977
Average	[a] 1.104	.561	.751
	[b] 1.100	.565	.751
Average for Groups 1 and 2	[a] 1.242	.674	.598
	[b] 1.226	.665	.598

[a] Data for cow 253 excluded. [b] Data for cow 253 included.

In Group 2 the data are somewhat different. The milk from every cow in this group for which complete data are available showed a decline of almost one-half in the fat-protein ratio from the ante-mortem to the first post-mortem, but an increase from the first to the second post-mortem milking. In the case of cow 811 the ratios could not be

determined, and in the case of cow 253 the ratio for the second post-mortem milk was not available.

LACTOSE

The percentage of lactose in the milk was determined by subtracting the sum of the percentages of fat, ash, and total protein from the percentage of total solids.[6] Variations in lactose percentages were less consistent than for the other constituents. In Group 1 there was a strong tendency for the lactose content to be high in the first and low in the second post-mortem milk. (Table 7.) The average percentage of lactose in the first post-mortem milk was 103.91, whereas that in the second post-mortem milk was only 87.81 per cent as high as that in the ante-mortem milk.

TABLE 7.—*Percentages of lactose in milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	4.50	4.71	4.06	104.67	90.22
292	4.80	5.20	4.27	108.33	88.96
123	4.85	5.15	4.32	106.19	89.07
272	5.05	4.87	4.19	96.44	82.97
Average	4.80	4.98	4.21	103.91	87.81
Group 2:					
846	4.37	4.20	3.65	96.11	83.52
903	3.71	3.52	2.97	94.88	80.05
811					
257	4.49	4.09	3.17	91.09	70.60
253	4.36	4.00		91.74	
443	4.83	5.05	4.34	104.55	89.86
255	4.15	4.21	3.24	101.45	78.07
Average	[a] 4.31	4.21	3.47	97.62	80.42
	[b] 4.32	4.18	3.47	96.64	80.42
Average for Groups 1 and 2	[a] 4.53	4.56	3.80	100.41	83.70
	[b] 4.51	4.50	3.80	99.55	83.70

[a] Data for cow 253 excluded. [b] Data for cow 253 included.

In Group 2, Table 7, the omissions for percentage of lactose are the same as for those of total protein in Table 5, and the averages are treated in the same manner. There was no upward trend in percentage of lactose from the ante-mortem to the first post-mortem milk, but the downward trend from the first to the second post-mortem milk was similar to that for Group 1. The percentage of lactose in the ante-mortem milk in Group 2 in general was lower than for Group 1. The lactose content of both the first and second post-mortem milk was not only lower for Group 2 than for Group 1, but was lower than that of the ante-mortem milk in Group 2.

In the combined averages for Groups 1 and 2, the first post-mortem milk appeared to be similar to the ante-mortem milk in percentage of lactose, but the second post-mortem milk was distinctly low in

[6] In subsequent analyses of ante-mortem milk and of the first and second post-mortem milks obtained from three other amputated udders, both the deduction method and the polariscope were used in determining the lactose content. A downward trend from ante-mortem to first and second post-mortem milkings was shown, and it was somewhat more pronounced than the average for the 11 cows herein reported. When the deduction method was employed the percentages of lactose in the first and second post-mortem milkings were, respectively, 99.0 and 75.0 per cent as high as in the ante-mortem, whereas the percentages of lactose as determined with the polariscope were, respectively, 93.1 and 67.1 per cent as high as in the ante-mortem milk.

lactose. By groups, the quantitative reductions in percentages of lactose in the milk from the first to the second post-mortem milkings did not differ materially, but the reduction from the ante-mortem to the second post-mortem was considerably greater in Group 2 than in Group 1. In the former case they are 0.77 and 0.74 per cent, respectively, for Groups 1 and 2. In the latter case they are 0.59 and 0.84 per cent, respectively, for Groups 1 and 2.

SUMMARY OF ANALYSES

A summary of the data in Tables, 1, 2, 3, 4, 5, and 7 is presented graphically in Figure 1. Six separate graphs show the relation of the percentages of total solids, butterfat, solids not fat, ash, total protein, and lactose in the milk obtained at the first and second post-mortem milkings, to those in the milk obtained at corresponding ante-mortem milkings. The 100 per cent line represents the average composition of the ante-mortem milk, with which all other analyses are compared.

QUANTITY OF BUTTERFAT RECOVERED FROM AMPUTATED UDDERS AT AND SUBSEQUENT TO THE FIRST TWO POST-MORTEM MILKINGS

The greatest difference between the ante-mortem and the post-mortem milk was in the percentage of butterfat. On an average, the butterfat test of the post-mortem milk was only about half as high as that of the ante-mortem milk. This low test, together with the fact that only about 70 per cent of the milk was recovered, could only result in the recovery of a relatively small quantity of butterfat.

Table 8 gives for each cow, (1) the average quantity of butterfat in the ante-mortem milkings on the two days immediately before slaughter; (2) the total quantity of butterfat in both post-mortem milkings; and (3) the relation which the latter bears to the former expressed in terms of percentage.

TABLE 8.—*Quantity of butterfat recovered from amputated udders in two post-mortem milkings, expressed as percentage of ante-mortem yield*

Group and cow No.	Average quantity of butterfat in ante-mortem milk	Quantity of butterfat in post-mortem milkings			Proportion of ante-mortem yield recovered in post-mortem milkings
		First	Second	Total	
Group 1:	*Pound*	*Pound*	*Pound*	*Pound*	*Per cent*
459	0.6629	0.4140	0.0171	0.4311	65.03
292	.6819	.0986	.0203	.1189	17.44
123	.5561	.1888	.0234	.2122	38.16
272	.8284	.1170	.0214	.1384	16.71
Average	.6823	.2046	.0206	.2252	[a] 34.34
					[b] 33.00
Group 2:					
846	.7477	.4158	.0067	.5125	68.54
903	.6357	.1737	.0572	.2309	36.32
811	.5444	.1602	.0138	.1740	31.96
257	.7451	.1500	.0432	.1932	25.93
253	.7918	.2636	.0493	.3129	39.52
443	.7902	.2370	.0948	.3318	41.52
255	.7764	.2794	.0429	.3223	41.51
Average	.7200	.2400	.0568	.2968	[a] 40.76
					[b] 41.22
Average for Groups 1 and 2	.7063	.2271	.0436	.2707	[a] 38.42
					[b] 38.33

[a] Average of percentages.
[b] Pounds recovered post-mortem divided by pounds recovered ante-mortem.

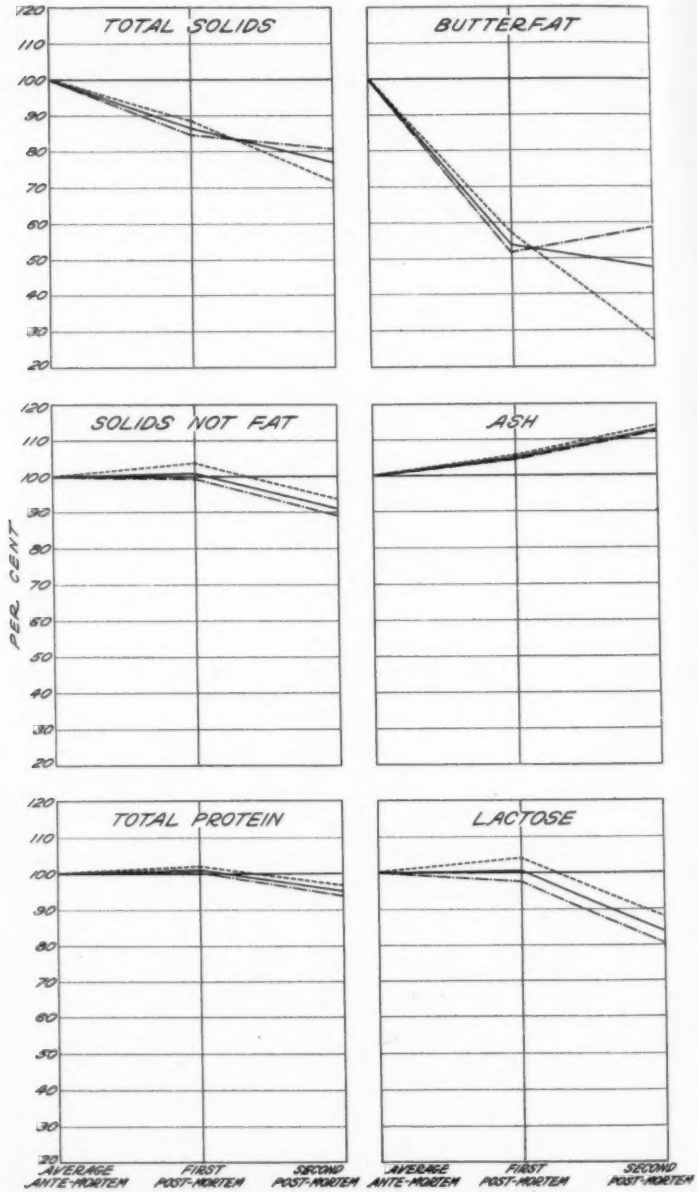

FIGURE 1.—Relative composition of the milk obtained from the udder before death of the cow, and in the first and second milkings of the amputated udder; expressed as percentage of ante-mortem composition. Dotted lines represent averages for cows of Group 1; broken lines represent averages for Group 2; and solid lines, for both groups combined

The low butterfat test and the consequent small quantity of butterfat recovered in the milk drawn from amputated udders, regardless of whether the udders were kept at blood temperature or allowed to become chilled, caused considerable speculation. Believing that a residue of butterfat might have remained in the amputated udder after post-mortem milking, the writers attempted to recover additional quantities through the use of solvents. It was believed that failure to recover fat in a solvent that had been injected through the teat into the secretory system of an udder and subsequently drawn out, should indicate that the tissues had not been affected and that the recovery of fat in solvents removed from lactating udders could be considered as butterfat.

The first preliminary trial was conducted with a nonlactating udder. Petroleum ether was introduced through the teats into the secretory system of two quarters of the udder. Removal of the solvent from one quarter was commenced five minutes after injection had ceased. From the other quarter the removal was commenced immediately. More than 2 gallons of the solvent was introduced into the two quarters, but after persistent manipulation of the udder only 1 pint, approximately, was recovered from each quarter. It appeared that, because of the volatility of this solvent, some of it permeated the tissues and escaped, but that the greater part was held within the finer divisions of the secretory system. Analysis showed that the 490 c c and the 488 c c of solvent recovered from the two quarters contained only 0.13 g and 0.19 g of fat, respectively.

Acetone was similarly introduced into the other two quarters of the same udder. Removal was commenced 9½ minutes after one quarter had been filled and two minutes after the other had been filled. In the former case, the teat became hard and nothing could be milked out until it was opened at the end. The fluid then came freely in a stream. Apparently the acetone attacked the tissue rather severely, as some of the lining of the teat canal was discharged in shreds. In the other quarter the action of the acetone appeared to be less severe, and the recovery of the solvent was more readily accomplished. The quantities of acetone recovered from the two quarters were 760 c c and 517 c c. They contained only 0.51 g and 0.74 g, respectively, of fat.

In two other trials hot water was pumped into amputated udders. Water at 70° C. was introduced into a rear quarter of a slightly lactating udder, and withdrawal of the water was commenced immediately after the udder was filled, which required 10 minutes. About 5,400 c c was introduced, but only 955 c c was recovered. Water at 70° apparently affected the tissues and caused a portion of the lining of the teat canal to slough off and come out as a plug. The front quarter of the same udder was filled with water at 40°. Five minutes was required to fill this quarter, and removal of the water was commenced at once. The quantity introduced was 3,700 c c, but only 425 c c was recovered. Water at 40° appeared not to injure the tissues. The fat contained in these two recoveries of 955 c c and 425 c c was 1.33 g and 0.49 g, respectively. It appears that water at 40° is about as effective as that at 70° in recovering butterfat, and is relatively less injurious to the tissues of the udder.

In another trial, water at from 40° to 42° C. was introduced into a nonlactating udder. Filling of the rear quarter required 4 minutes and removal was commenced immediately thereafter. Filling the front

quarter required 3½ minutes and removal of the water was commenced 1½ minutes later. The quantity introduced was not measured. The quantity recovered from the two-quarters was mixed together and amounted to approximately 100 c c. It contained only 0.001 g of fat.

In an additional trial with a cow secreting a small quantity of milk, the udder was emptied as thoroughly as possible by hand milking and by inserting into the teat a milking tube attached to a vacuum pump. About 135 c c of milk was removed from the two left quarters in this manner. Approximately one-half gallon of petroleum ether was then pumped into the left front quarter. The vacuum pump was again attached to a milking tube in this quarter and 470 c c of the solvent was removed. Analysis indicated that this recovered solvent contained a total of 4.376 g of fat. As the specific gravity of the petroleum ether was 0.632, the solvent recovered contained approximately 1.47 per cent of fat by weight. As an indication of the relative efficiency of hand manipulation of the udder and of similar manipulation aided by a vacuum pump, this test is of interest. In the first trial with petroleum ether and removal by hand, about 13 per cent of the solvent was recovered. When the vacuum pump was used approximately 25 per cent was recovered. The fact that the first trial was made with a nonlactating udder, and the second with a slightly lactating udder, may have influenced these results.

After the solvent had been removed (by vacuum) from the left front quarter, 3,370 c c of cold water was pumped into the left rear quarter. By the use of the vacuum pump together with hand manipulation, 733 c c of the water was drawn out. On again comparing the efficiency of the hand method with the vacuum method, it appeared that when water was pumped into the udder, withdrawal by hand in two trials recovered 17.7 and 11.5 per cent of the quantity introduced. The vacuum method of removal recovered 21.8 per cent. All three tests were conducted with udders secreting small quantities of milk.

These preliminary trials indicate (1) that petroleum ether as a solvent does not dissolve the tissue fat to any significant extent, and that its action on the udder tissue is less severe than that of acetone; (2) that the use of the vacuum pump in combination with hand manipulation is more efficient than hand manipulation alone in recovering from the udder the fluids previously injected into it; and (3) that not more than about one-fourth of the fluid injected was recovered by any method. The small recovery of fluid injected into the secretory system of the udder leads the writers to believe that a considerable quantity of milk may be left within the udder after the amputated udder has been milked out. This observation also strengthens the belief that nearly all the milk obtained at any milking is present in the udder at the time the milking process is commenced, even though the percentage recovered in some cases is less than in others and may be considerably less than 100 per cent of the quantity obtained at corresponding milkings before death.

As petroleum ether appeared to be a relatively efficient solvent for butterfat and comparatively nondestructive to udder tissues, it was selected for use in an attempt to recover additional butterfat from three amputated udders that had been subjected to two post-mortem milkings four hours apart. Before the solvent was introduced, the udder of cow 257 was subjected to a third post-mortem milking,

which yielded 0.4 pound of milk. Assuming that this milk contained 1.3 per cent butterfat, which is the average of the tests of the second-post-mortem milking from the two halves, the quantity of fat recovered was 0.0052 pound. Petroleum ether then was pumped through the teat into the right rear quarter for six minutes, and into the right front quarter for five minutes. Removal from each quarter was commenced within two minutes after the completion of the injection. Removal in this instance was entirely by hand manipulation. The quantity recovered from the two quarters was not measured, but it was found to contain 20.135 g of fat. Assuming that the other half of the udder would have yielded an equal amount of fat, the fat recovery in the solvent for the udder would have been 0.0888 pound.

The left half of the udder of cow 253 was similarly treated. A third post-mortem milking of the udder yielded 0.13 pound of milk containing 2.52 g, or 0.0056 pound of fat. Into each of the left quarters injection of petroleum ether was continued for three minutes. Removal was commenced at once. Hand manipulation was followed by application of the vacuum pump, and the total recovery of solvent appeared to be about half as much as the quantity introduced. The solvent from two quarters of the udder contained 2.225 g of fat, indicating a probable recovery in the solvent of 4.45 g or 0.0098 pound from the udder.

The experiment was repeated with the udder of cow 443. A third post-mortem milking yielded 0.36 pound of milk containing 10.75 g or 0.0237 pound of fat. Petroleum ether was introduced into the two left quarters. Injection was continued for two minutes in each quarter. Hand manipulation and employment of the vacuum pump resulted in the recovery of 1,700 c c, or more than 43 per cent of the quantity injected. The solvent which was recovered contained 20.33 g of fat, indicating a probable recovery of 40.66 g or 0.0897 pound of fat from the entire udder.

If it be assumed that an amount of butterfat equal to that recovered in corresponding ante-mortem milkings was present in the udder of the cow when killed, the third post-mortem milking and the use of a solvent resulted in the recovery of 0.94 and 16.09 per cent, respectively, of the amount of butterfat calculated to be retained in the udder of cow 257 after completion of the first and second post-mortem milkings. Corresponding recoveries in the third post-mortem milking and in solvents were 1.17 and 2.05 per cent, respectively, for cow 253, and 5.07 and 19.19 per cent, respectively, for cow 443. The retention of the butterfat in the udder, after all attempts to recover it had been made was, on this basis, 0.46, 0.46, and 0.35 pound, respectively, for the three cows, which was equivalent to 61.45, 58.54, and 44.29 per cent, respectively, of the total quantity of butterfat present when the cow was killed.

A fairly definite idea of the effectiveness of an additional post-mortem milking followed by the use of a solvent, in recovering residual fat from amputated udders after two post-mortem milkings, may be obtained from Table 9.

TABLE 9.—*Recovery of butterfat subsequent to the second post-mortem milking of amputated udder*

Cow No.	Butterfat in ante-mortem milk	Total butterfat in milk obtained at first and second post-mortem milkings	Proportion of ante-mortem yield recovered in first and second post-mortem milkings	Butterfat recovered in third post-mortem milking	Fat recovered in solvent	Total fat recovered from all sources	Proportion of ante-mortem fat yield recovered from all sources
	Pound	*Pound*	*Per cent*	*Pound*	*Pound*	*Pound*	*Per cent*
257	0.7451	0.1932	25.93	0.0052	0.0888	0.2872	38.55
253	.7918	.3129	39.52	.0056	.0098	.3283	41.46
143	.7992	.3318	41.52	.0237	.0897	.4452	55.71
Average	.7787	.2793	[a] 35.66			.3536	[a] 45.24
			[b] 35.87				[b] 45.40

[a] Average of per cent values.
[b] Obtained by dividing total pounds of fat recovered by total pounds of fat in ante-mortem milk.

On an average, 35.66 per cent of the quantity of fat contained in the ante-mortem milk from these three cows was recovered in the first two post-mortem milkings. By adding a third post-mortem milking and subsequently employing a solvent, the butterfat recovery was increased from 35.66 per cent to 45.24 per cent of the quantity contained in the ante-mortem milk. The average post-mortem recovery of milk for the same cows was 74.03 per cent. Corresponding percentages, obtained by dividing the total number of pounds of butterfat recovered in the first, second, and third post-mortem milkings and in the solvent, by the average number of pounds of butterfat in the ante-mortem milkings, are 35.87 and 45.40, respectively.

SUMMARY AND CONCLUSIONS

The butterfat test of the milk obtained from amputated udders was, on an average, about half as high as that of the milk obtained from the same udders before death.

Keeping the udder at approximately blood temperature until after the completion of post-mortem milking had a distinct tendency to keep the butterfat test of the milk from the second post-mortem milking as high as, or slightly higher, than that of the milk from the first post-mortem milking. In the experiments with the cows in Group 1, where post-mortem temperatures of the udder were not controlled, the butterfat test was only about half as high in the first post-mortem milking as in the ante-mortem milking, and only approximately half as high in the second as in the first post-mortem milking. In the experiments with the cows in Group 2, where post-mortem temperatures of the udder were controlled, the butterfat test of the first post-mortem milking was only about half as high as that of the ante-mortem milking, but the milk obtained at the second post-mortem milking was higher than that of the first post-mortem milking in butterfat test.

All measures introduced for the purpose of controlling the conditions under which the cow was killed, or under which the udder was held after death, failed to increase the total recovery of butterfat in the post-mortem milking to more than about 40 per cent of the total butterfat yield that was secured in the ante-mortem milking. Keep-

ing the udder under controlled conditions resulted in the recovery of somewhat more butterfat in the second post-mortem milking than was the case when the udder temperatures were not controlled, but this increased recovery was not sufficient to increase materially the total amount recovered. Thus the percentage of butterfat in the milk was maintained and even slightly increased from the first to the second post-mortem milking in Group 2, where the udders were kept at blood temperature. The cause of the consistent decrease of nearly one-half in the butterfat test of the first post-mortem as compared with the ante-mortem milk has not been determined. (See reference to the suggested possible effect of the nervous shock produced by killing the cow and severing the nerves in the process of amputating the udder (*8*).)

Flushing out the secretory system of amputated udders with a solvent increased to some extent the total quantity of butterfat recovered. In trials with three udders, the addition of a third post-mortem milking, and a subsequent injection and removal of petroleum ether, increased the proportion of the total butterfat obtained from amputated udders, from 35.87 to 45.40 per cent of the quantity of butterfat in the milk obtained from the same udders at corresponding milkings before death.

If it be assumed that the amount of butterfat present in the udder when the cow was killed was equal to that recovered in corresponding ante-mortem milkings, the retention of butterfat in the three udders after three post-mortem milkings and subsequent flushing with petroleum ether, would be 0.46, 0.46, and 0.35 pound, respectively. These quantities are equivalent to 61.45, 58.54, and 44.29 per cent, respectively, of the total quantity of butterfat present when the cow was killed.

Not more than 44 per cent of the fluid injected into amputated udders was subsequently removed in any one of several trials, even when hand manipulation was combined with the use of a vacuum pump.

Neither the quantity of milk recovered from amputated udders nor its relative test were as high as those shown by Petersen and his associates (*5*). This recalls a previous comment (*8*) regarding the possible effect of milking one-half of the udder immediately before death, on the recovery of milk from the other half after death.

The percentage of total solids was distinctly lower in the first post-mortem than in the ante-mortem milk, and still lower in the second than in the first post-mortem milk. When the amputated udders were kept warm the decline in percentage of total solids from the first to the second post-mortem milking was greatly reduced. Since the butterfat is included in the total solids, such a tendency is to be expected.

The percentage of solids not fat was nearly the same in the first post-mortem as in the ante-mortem milk, but was distinctly lower in the second than in the first post-mortem milk. Controlling the temperature of the udder did not prevent the reduction in the percentage of solids not fat between the first and second post-mortem milkings.

The percentage of ash was higher in the first post-mortem than in the ante-mortem milk, and decidedly higher in the second than in the first post-mortem milk. This trend is shown for every cow in each

group and does not appear to have been affected by the temperature under which the udder was held after it was amputated.

The percentage of total protein was almost the same in the first post-mortem as in the ante-mortem milk, but it was lower in the second post-mortem than in either the ante-mortem or the first post-mortem milk. This trend is not so consistent in the individual experiments as in the case of ash, but averages almost the same regardless of the temperature at which the udder was held after amputation.

The fat-protein ratio, as anticipated, followed a trend very similar to that of the butterfat test. In Group 1 the average ratio for the first post-mortem milk was only slightly more than half of the average ratio for the ante-mortem milk. The ratio for the second post-mortem milk was almost exactly half of that for the first post-mortem milk. In Group 2 also, the reduction in ratio from ante-mortem to first post-mortem milk was about one-half, but the ratio for the second post-mortem milk was decidedly higher than that of the first post-mortem milk. The average fat-protein ratio for the first post-mortem milk in Groups 1 and 2 combined was slightly more than half that of the ante-mortem milk, whereas the ratio for the second post-mortem milk was slightly less than half of that for the ante-mortem milk.

When the udders were allowed to become chilled, the percentage of lactose was slightly higher in the first post-mortem milk and much lower in the second post-mortem milk than in the ante-mortem milk. When the udders were kept warm after amputation, the trend was almost the same, except that the percentage of lactose in the first post-mortem milk was slightly below instead of above that of the ante-mortem milk. When Groups 1 and 2 are combined, the percentage of lactose in the first post-mortem milk was almost the same as that in the ante-mortem milk, but the second post-mortem milk had a much lower percentage than the ante-mortem milk.

The results based on 11 experiments confirm those based on the first two (7), and show that the most outstanding difference between the milk obtained from the udder of the living cow and that obtained from the amputated udder of the same cow after death was in the butterfat test.

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THE ORIGIN, DEVELOPMENT, AND INCREASE OF CHLOROPLASTS IN THE POTATO[1]

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INTRODUCTION

Much work has been done on mosaic and chlorosis, diseases whose primary visible effect is on the chloroplasts of the host plant. Yet a thorough search of the literature brings to light very few references to the normal chloroplast of the potato (*Solanum tuberosum* L.) or of any other member of the Solanaceae. Every phase of the life history of normal chloroplasts has been described again and again in other plants, but these data when brought together appear to form a chaotic mass of irreconcilable facts. The question arises which, if any, of these data apply to the normal chloroplasts of the potato or other members of this group. Obviously no progress can be made in the study of diseased chloroplasts until a firm basis is laid in a knowledge of the healthy normal ones.

Though small, the nightshade family includes many plants of economic importance, such as the potato, tomato, pepper, eggplant, tobacco, and petunia. All these plants are affected by degeneration diseases of the chloroplasts. Since little work has been done on any of these plants, the potato was considered a suitable and representative member of the group in which to study the formation and division of healthy chloroplasts.

HISTORICAL REVIEW

Since the discovery of plastids in 1791, investigators have striven to settle the more important phases of their history, i. e., their origin, structure, and method of division.

There are three outstanding theories as to the origin of chloroplasts.

(1) Chloroplasts arise by the condensation and contraction of the cytoplasm.

(2) Chloroplasts arise from preexisting bodies. Schimper (*25*, *26*, *27*)[2] was able to trace a complete succession of stages in the life history of chloroplasts. Mottier (*18*) was aware of the fact that chloroplasts develop from small bodies but believed that these bodies were merely certain stages in the continuous life history of individual chloroplasts.

(3) Chloroplasts arise from chondriosomes. When the study of plastid origin was extended to the higher plants a new school was originated by Guilliermond (*4*). There can be no doubt as to the accuracy of the observation of the supporters of the chondriosome and primordia theories of plastid origin. The explanation of the variance of the results probably lies in the different types of material used. Many investigators have used water plants. Others have

[1] Received for publication Dec. 7, 1931; issued October, 1932.

[2] Reference is made by number (italic) to Literature Cited, p. 433.

used mosses, liverworts, and ferns. Much of the work on the higher plants has been confined to the roots, but these organs, of course, are not a suitable place to observe chloroplast origin and so can not be used for comparison. In reproductive organs the chloroplasts are not functioning as in a leaf. Thus the dissimilarity of the plants and the plant organs studied may account for the widely divergent results not only of the present investigation but of those which led to the formation of the chondriosome and primordia theories.

Gris (*3*), who, so far as the writer is aware, is the only investigator who has worked on the origin of the chloroplasts in the potato leaf, described the differentiation of chloroplasts in the cells of leaves of *Solanum tuberosum* and various other plants, such as *Hydrangea hortensia, Lilium album,* Acanthophippium, Aucuba, Sempervivum, and Vanilla. In the very young hexagonal cells of the potato leaf he found the nucleus surrounded by a green, granular network. In older leaves the cells were entirely filled with granular matter. This granular substance broke up into sections, at first polyhedral but later assuming the spherical shape of normal, mature chloroplasts. Gris also found that development did not take place simultaneously throughout the entire leaf cross section, but that all stages in their differentiation from the cytoplasm might be observed within a limited area.

No record has been found of any attempts to trace the development of chloroplasts from chondriosomes or primordia outside the cell in cell sap. In fact Prát (*19*) and Börger (*2*) considered plant juice unsuited to the culture of isolated tissues and cells. Kny (*10*) and Heitz (*6*) were able to observe activity in isolated tissues, the latter using Knop's solution. Lubimenko (*13, 14, 15*) and Priestley and Irving (*20*) isolated plastids, the latter in cell sap. In this case there was some manufacture of sugar.

Belzung (*1*) investigated greening in potato tubers and was convinced that the starch grains formed the chlorophyll-impregnated areas of the cells. He was also of the opinion that these bodies are not true chloroplasts in that they do not manufacture starch.

At least three methods of plastid division have been noted. That most frequently observed is a process of constriction or pinching. Mikosch (*17*) described a type whereby the color concentrated at the poles and the ends grew away from each other. Schmitz (*28*) observed distinct fission. Kozlowski (*11*) believes the so-called division stages are merely accidental pairings of chloroplasts.

ORIGIN AND DEVELOPMENT OF CHLOROPLASTS

ATTEMPTS TO CULTIVATE PLASTIDS

In an effort to cultivate plastids plant juice expressed from actively growing portions of young plants by a metal press was filtered through several layers of cheesecloth, filter paper, and infusorial earth. The filtrate then passed easily through a Jenkins bacteriological microfilter. The filtrate contained no bodies which could be detected with the microscope. It was kept in the diffused light from a south window. The liquid remained clear and uncontaminated indefinitely. The nonappearance of bodies was probably due to one of two things. Either the culture medium was unfavorable for the development of the primordia, or the primordia are too large to pass through the

Jenkins filter. Under these conditions these results alone do not justify the assumption that chloroplasts do not arise from primordia.

GREENING OF TUBERS EXPOSED TO LIGHT

Detached tubers were greened by exposure to light in a window. The coloration extended into the tubers for some depth, but most attention was given to cells directly beneath the corky layer.

Colorless cells contained numerous large starch grains. In green cells starch was present, but the grains were often capped at one end by a mass of green protoplasm of varying size. The size of these caps was inversely proportional to the size of the accompanying grain, that is, the smaller the starch grain the larger the accompanying green area. The green patch was merely a rim, a considerable mass, or in some cells entirely inclosed the smaller grains in a green sheath. Besides these green areas accompanying the starch other detached green masses of rather indefinite outline were observed. (Pl. 1, A and B.) The size of the starch grain caps and the independence of some of the green masses make it improbable that this process is a conversion of leucoplasts into chloroplasts. Leucoplasts are soon stretched by grains of storage starch to thin films. The independent green masses can hardly be thought of as transformed leucoplasts, nor do they have the structural characteristics of chloroplasts. They seem to be regions in the cytoplasm which have become green, i. e., in which the chlorophyll has developed under stimulus of light. This phenomenon was described as early as 1877 by Wiesner (*29*) as "chlorophyll tinging the cytoplasm," and Harper (*5*) has called attention to the fact that a delimiting membrane is not essential in a chloroplast.

Some cells entirely devoid of starch contained rather definitely formed green bodies, vacuolate, and of varying size and shape, although in general resembling each other. (Pl. 1, C.)

Cells from the ungreened inner layers contained intact starch grains. Cells from the next region toward the surface contained corroded starch grains, broken in fragments, yet retaining the original shape. In the next layer starch fragments were smaller and fewer, and for the most part contained in a colorless vesicle. Directly beneath the cork the cells contained very small similar structures, but the colorless vesicles were here replaced by green regions inclosing particles of starch. (Pl. 1, E.)

Greening in the cytoplasm of the cells beneath the cut exposed surface of a tuber is due to large starch grains being partially enveloped by green protoplasmic masses. The starch grain was often found lying in a large vesicle inclosing also the green mass of protoplasm. (Pl. 1, F.) In some instances the green area assumed the shape of definitely formed bodies with one large or many small vacuoles, sometimes so large and numerous as to burst the outer green covering. (Pl. 1, D.)

Guard cells on small tuberlike sprouts exposed to light contained green bodies, evidently filled with starch; similar bodies from tuberlike sprouts grown in the dark were colorless although containing starch. (Pl. 1, G and H.)

Pith cells from fleshy sprouts grown in the light contained bodies which resembled normal chloroplasts in form, size, and color; cells

from sprouts kept in the dark contained only colorless bodies with minute starch grains. (Pl. 1, I and J.)

Guard cells of leaf buds had a uniform green cytoplasmic coloration with no suggestion of concentration into restricted regions and no apparent relation to the starch grains. (Pl. 1, K.)

The wide variety in shape and structure of the green bodies in tubers and sprouts exposed to light discourages the idea of discovering from them any facts concerning the origin of normal leaf chloroplasts. Perhaps they are not really chloroplasts at all. (1) Their shape is variable, indefinite, and not sharply outlined. Chloroplasts are oval or spherical and sharply defined. (2) Their size is not constant; even in the same cell bodies vary within a wide range. Normal chloroplasts usually have a constant definite size for a given species. (3) Their structure shows a lack of organization. They often differ little in density from the surrounding cytoplasm, their green color serving as their only distinction. Chloroplasts give the impression of definite organization with the ability to perform certain functions. (4) No divisions of these bodies were found in any of the material examined. In the life history of normal plastids divisions may be observed under a variety of conditions. (5) Although some green areas contained vacuoles and starch grains they seemed to be only temporary organs whose functions would soon cease or be taken over by more efficient bodies, i. e., by the true chloroplasts of the plant proper.

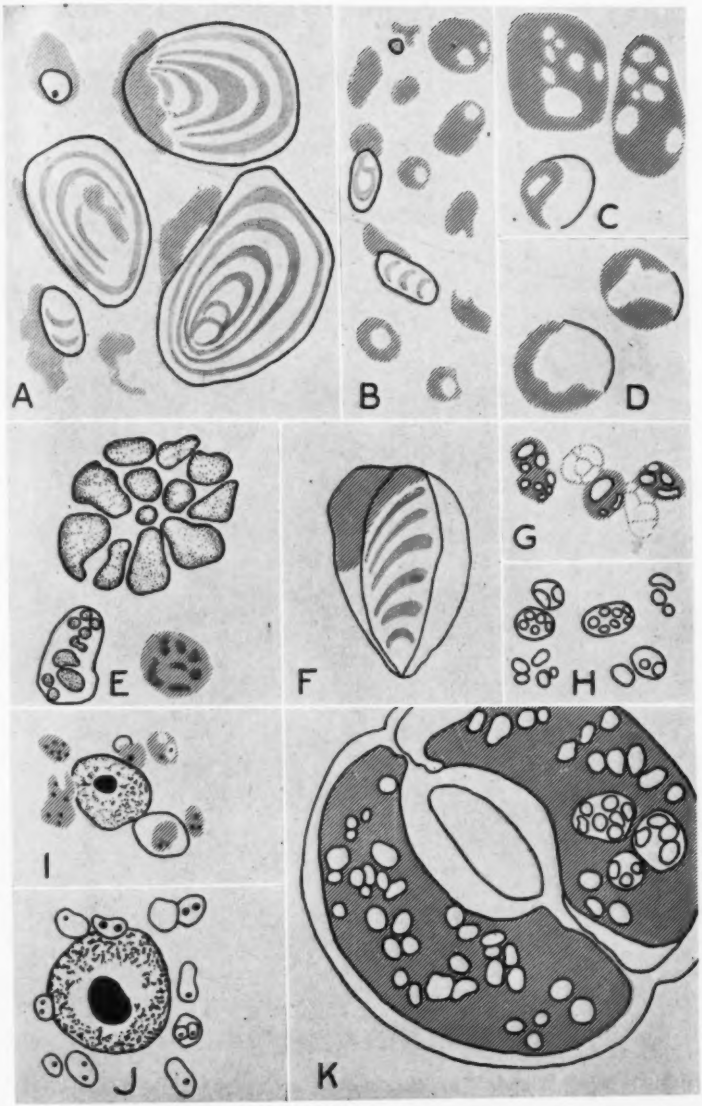
CHLOROPLASTS IN THE LEAVES

Methods

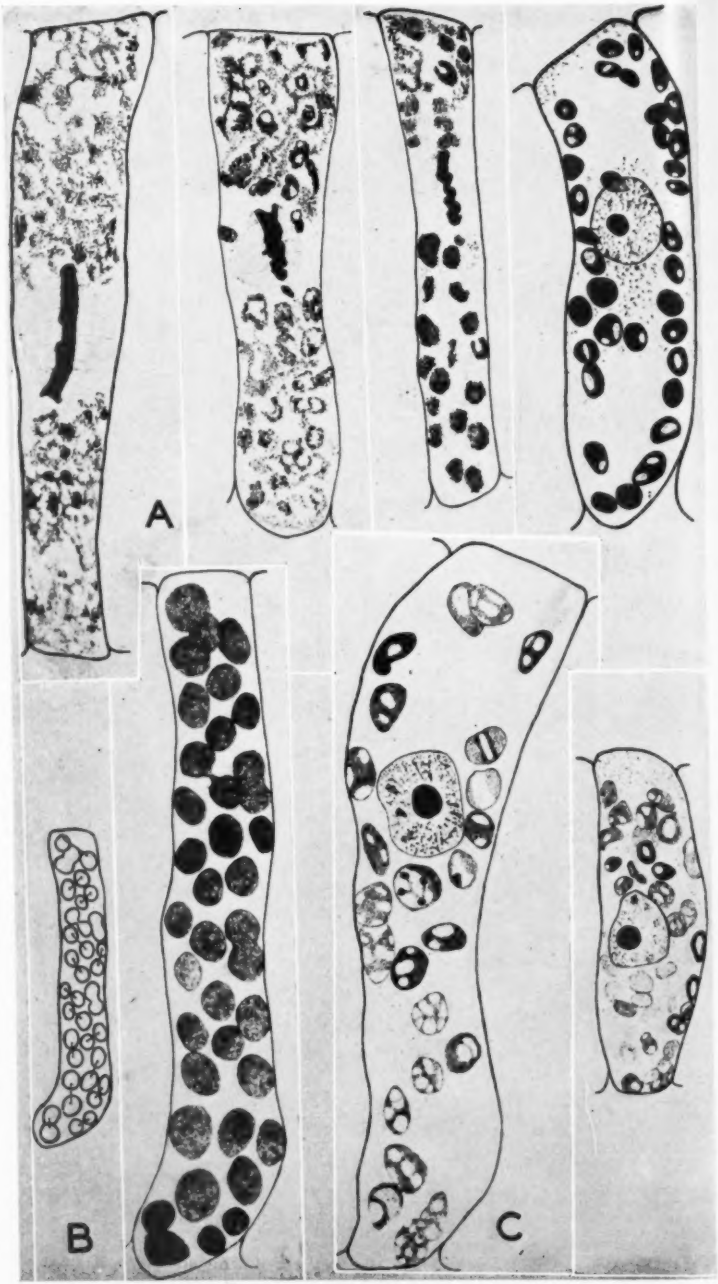
Material for the study of the origin and division of chloroplasts in potato leaves was obtained from plants grown in the greenhouse and out of doors. Fixations of leaves of different sizes from plants 1 to 4 inches high were made in Fleming's medium at all hours of the day and night in good growing weather. Sections 4 to 10μ thick, according to the age of the leaf, were stained in either Flemming's triple or Heidenhain's iron-alum haematoxylin stains. In a search for development of chloroplasts from chondriosomes material was fixed in Benda's and Altmann's fixing solutions, as well as in Flemming's medium, and stained in Altmann's aniline-fuchsin or in iron-alum haematoxylin. Fixing fluids which contain acetic acid are claimed to be destructive to chondriosomes. Rudolph (*22*, *23*), with a basic solution and a varying amount of acetic acid, found chondriosomes to be uninjured by concentrations of acetic acid equivalent to that in Flemming's solution. The results obtained with Altmann's or Benda's fixing fluids were quite unsatisfactory. The cells fixed in Altmann's fluid lacked any semblance of detail or differentiation. Benda's solution caused the chloroplasts to appear badly misshapen without the detail obtained by Flemming's fixing fluid followed by the iron-alum haematoxylin stain.

Formation of Plastids

Formation of true plastids in the leaves differs greatly from that of the green bodies in exposed tubers. In stained sections of very young leaves, taken from the innermost part of growing tips, the cell cytoplasm appeared homogeneous and very dense. These leaves



Bodies from potato tubers and sprouts exposed to light or kept in dark. All × 1,450. Shading represents green coloration. A to C, Green masses of cytoplasm from cells directly beneath skin of potato tuber exposed to light several days. D, Same as above, but from cells beneath new skin formed over cut surface. E, Bodies from three different depths of exposed tuber showing transition from disintegrating starch grain in deeper cell to greened body inclosing fragments of starch in cell directly beneath skin. F, Starch grain inclosed in vesicle and accompanied by green mass from new skin formed over cut surface of tuber exposed to light many days. G, Green bodies from hydathode of tuberlike sprout kept in light many days. H, Same from tuber kept in dark. I, Green bodies from pith cell of petiole of sprout exposed to light; starch stained with iodine. J, Same from sprout kept in dark. K, Stoma from leaf on young sprout, exposed to light; note general diffusion of chlorophyll throughout cytoplasm



A, Palisade cells showing development of plastids from vacuolated cytoplasm to mature bodies. × 2,900, 10 p. m. fixation. B, Palisade cell containing 11 plastids in the process of division and the rest in a very distinct pattern of pairs (right). × 2,900. Plan of plastid divisions and pairing (left). × 1,300, 6 a. m. fixation. C, Palisade cells from old leaf on left and young leaf on right showing variation in size of cells and plastids. No cytoplasm apparent in older cell and plastids more vacuolate than in the young cell. In young cell some plastids are just emerging from the ground cytoplasm. × 2,900, 6 p. m. and 1 p. m. fixation

were green, so it may be assumed that the green coloring matter was diffused throughout the cell contents, and examination of fresh material confirmed this. Sections of very small fresh leaves show no oval plastids, nor, in fact, any definite bodies, but only a fairly uniform, dense, green cytoplasmic mass. The cytoplasmic chlorophyll gathers into specific delimited regions.

In slightly older cells many small vacuoles accompany regional changes in cytoplasmic density and give evidence that the next step in plastid formation is the cutting up of the chlorophyll-impregnated cytoplasm. Vacuoles appear not only between the masses but within them as well. As the cells mature the cytoplasm concentrates, first in irregular patches, then in definite, regular, uniform shapes. During the rounding off masses are often held together for a time by cytoplasmic connections which disappear as the plastids become fully formed. Even in its very earliest form, as a shapeless mass of cytoplasm slightly denser than the surrounding cytoplasm, the chloroplast "anlage" is of considerable size. The process is one of concentration and contraction rather than of expansion and growth, so these original patches may be even larger than the resulting fully developed chloroplasts. (Pl. 2, A.)

Plate 3 shows development of chloroplasts in palisade cells. The young embryonic cell (A), short in proportion to its width, and with the nucleus taking up a large part of the contents, shows homogeneous cytoplasm, although a few tiny vacuoles may appear. These vacuoles become more pronounced in slightly older cells. B shows the two larger vacuoles in the ends of the cell, which are very characteristic of cells at this stage. The cytoplasmic knots are becoming more distinct and are taking on the appearance of immature plastids. These plastids (C and D) condense into the mature organs. There is no evidence of nor need for the intervention of any bodies, either chondriosomes or primordia; the whole process appears to be simply a cutting out of chloroplasts from the homogeneous cytoplasm. The plastids arise directly from cytoplasmic regions impregnated with chlorophyll.

In practically all the cells shown in Plate 3 the nucleus is in the process of dividing. There is no direct relation between plastid development and nuclear division; both processes take place in the early life of the cell. In the nearly mature palisade cells (D) a few plastids appear to be elongated as though about to divide, and many contain starch grains.

Differentiation of Plastids in Different Parts of the Leaf

Plastid differentiation in the cells of the spongy tissue of the potato is similar to that in the palisade parenchyma. (Pl. 4.) Large vacuoles appear and very dense regions of the cytoplasm can be easily distinguished (A). In B a large vacuole and in one section of the cell at least three distinct chloroplasts appear. In C and D the chloroplasts are well differentiated and contain starch grains. Some are rather elongated and either have not yet rounded off or are about to divide. The chloroplasts of Plate 8, B, are mature with sharp definite outlines and take a dense black stain. In Plate 4, C, and D, and Plate 8, B, many chloroplasts are surrounded by conspicuous clear zones. The nuclei of these spongy parenchyma cells are in

some phase of mitosis. Divisions occur in all planes. In most cases the distribution of the plastid material seems to be such that the new cells resulting from this division receive equivalent amounts.

Differentiation of the cytoplasm is not simultaneous throughout the leaf. In younger leaves the midrib is larger in proportion to the leaf lamina than in older leaves. Rib cells show rounded mature chloroplasts while the lamina cells contain only homogeneous protoplasm or early stages of breaking up. Spongy parenchyma cells show differentiated plastids before the palisade cells. Although spongy parenchyma plastids develop more rapidly than palisade plastids, differentiation is not complete throughout the spongy tissue before it starts in the palisade cells. Consequently a leaf section may show patches of densely stained mingled with patches of faintly

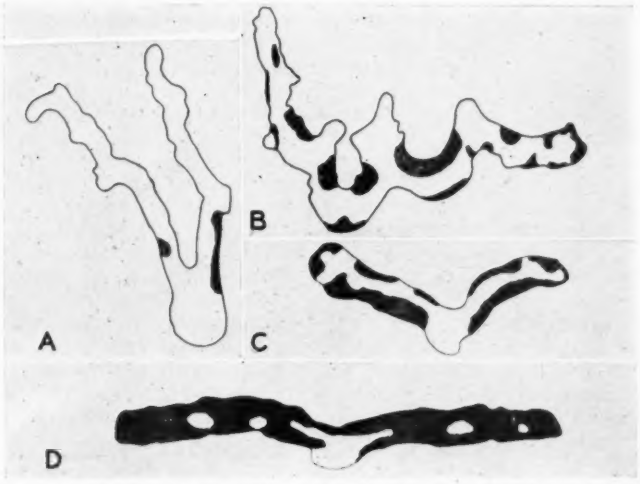
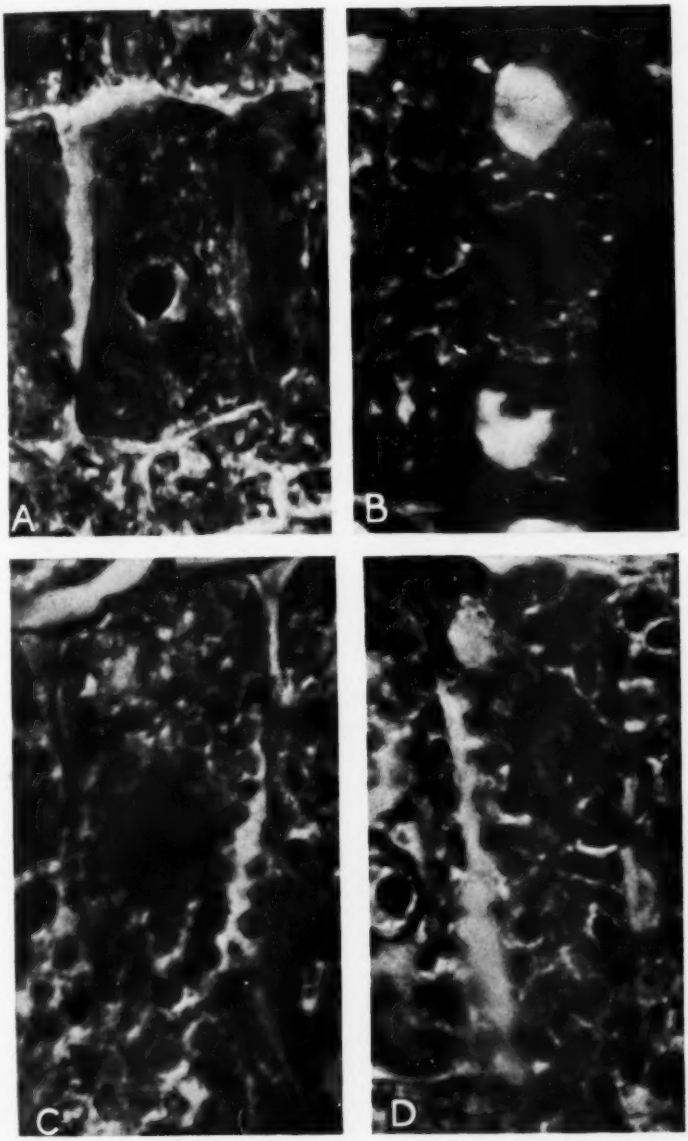


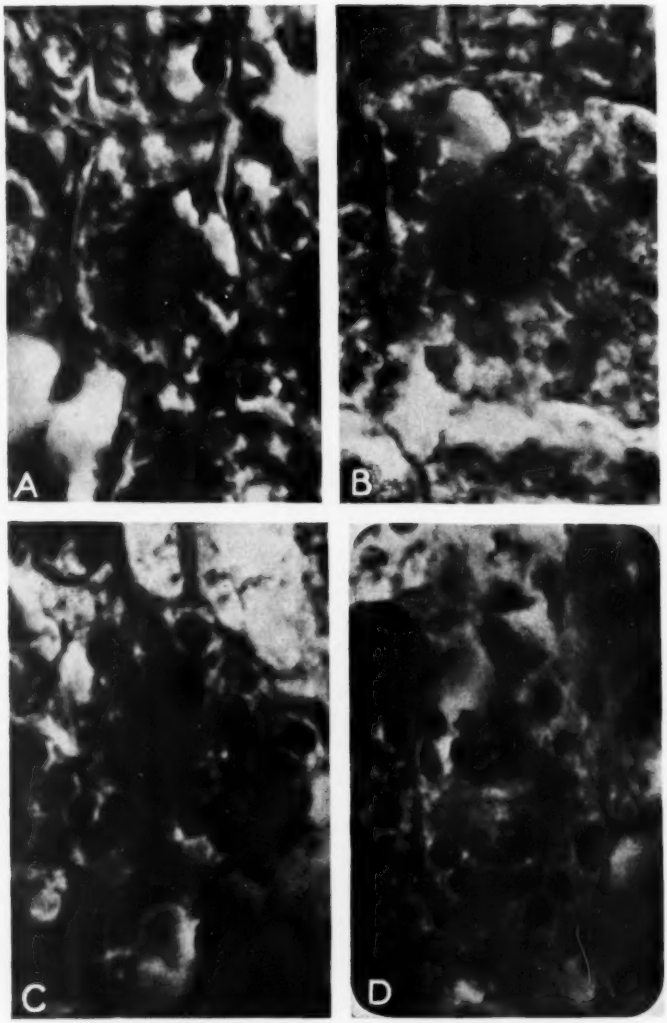
FIGURE 1.—Diagrammatic cross sections of leaves showing progressive differentiation (in order lettered) of plastids (in black). × 35

stained plastids within both spongy and palisade tissues. The spongy tissue is filled with densely stained plastids while the palisade parenchyma still contains patches of faintly stained ones. (Fig. 1.) Even within individual cells differentiation of plastids occurs first in certain regions and not uniformly throughout the whole cell. Usually plastids nearer the epidermis or around the cell wall stain more densely than those of the lower half or center of the cell. (Pls. 5 and 6.)

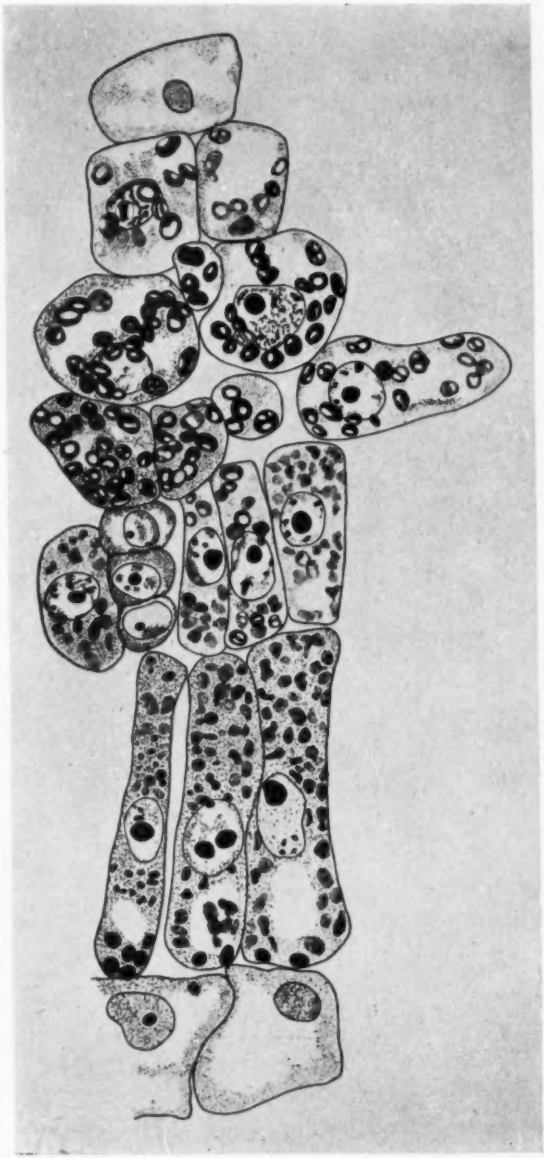
In the midrib fully developed plastids often contained starch grains so large that the chloroplast itself was stretched to a mere shell surrounding the starch. (Fig. 2.) In both spongy and palisade tissues plastids were of two sorts, one densely staining, uniform throughout or containing a few small starch grains, the other indefinitely outlined and faintly staining. (Pl. 6.) In the two rows of palisade cells the nuclei are prominent but the chloroplasts are very indistinctly defined; in the upper row they appear as denser portions



Stages in the process of plastid differentiation in palisade cells. × 2,400. A, Embryonic cell, cytoplasm filled with many small vacuoles, 3 a. m. fixation. B, A large vacuole at either end, cytoplasm concentrating in dense irregular patches, 4.30 a. m. fixation. C, Plastids somewhat more rounded, 12.30 p. m. fixation. D, Plastids nearly complete, some containing starch grain 10 p. m. fixation



Stages in the differentiation of plastids in cells of spongy tissue. × 2,400. A, Embryonic cell, slightly vacuolate, 4.30 a. m. fixation. B, Appearance of one large vacuole, three distinctly outlined plastids evident, the rest of the cytoplasm contracting in dense masses, 9 p. m. fixation. C, More mature cell; plastids definite, some containing starch, 9 p. m. fixation. D, Same as C, 12.30 p. m. fixation



Section of a young leaf showing variation in degree of differentiation of plastids in spongy tissue and palisade cells, 11 p. m. fixation. × 2,900

Photomicrograph of section through young leaf showing variation in degree of differentiation of plastids in spongy tissue and palisade cells, 10 p. m. fixation. × 1,100

of the cytoplasm with no definite shape; in the lower row they are somewhat more distinct. None retain the stain but appear as faint gray patches. The chloroplasts of the spongy parenchyma stain very densely, as intensely black, regular bodies, distinctly independent of the surrounding cytoplasm, in some instances apparently undergoing division. These cells are less compactly filled with plastid material than are the palisade cells, and the nuclei are less prominent. The palisade cells reach their normal mature state more slowly than do the cells of the spongy tissue. Zirkle's (*31*) similar variation in plastid-staining reaction in striped (variegated) corn was ascribed to the fact that some plastids were degenerated or permanently undeveloped, while others were normal, the uneven staining not being an indication of stages in progressive development. Ma (*16*) attributed this variation in the staining reaction in Isoetes to the condition of the starch within the plastid, that is, whether starch was being formed or hydrolized. The staining reaction of potato plastids seems rather to depend on the degree of development of the plastids themselves. If this reaction were dependent on the formation and hydrolysis of starch, it would recur with some regularity and frequency. No explanation can be given of this irregular progress of the development of the plastids in the potato-leaf cells.

FIGURE 2.—Shell-like chloroplasts of midrib. × 4,200

CHLOROPLASTS AND CHONDRIOSOMES

The description which has just been given of the development of the leaf chloroplasts leaves no place for the theory of their growth from small bodies in embryonic cells. Since the chondriosome has acquired such importance in the recent literature on chloroplast formation, special attention was given to the sections with chondriosomes in mind. No bodies resembling chondriosomes have been observed. Some investigators have found these bodies not only in young cells where chloroplasts were forming but also, in smaller numbers, in mature cells. Even in the youngest potato cells no chondriosomes were seen. The cytoplasm appeared homogeneous, granular, but free from rods, spheres, and threads. Young, recently differentiated, irregular plastids are somewhat smaller than those found in old leaves, but never so small that they would be mistaken for chondriosomes. The smallest chloroplasts seen were no less than 3μ in diameter; chondriosomes do not exceed 1μ in diameter.

RELATION OF NUCLEAR DIVISION TO PLASTID DIFFERENTIATION

The nucleus divides in the very young cell; at the same time the plastids differentiate in the cytoplasm; the two processes, while coincident, have evidently no relation to each other. Plastid differentiation extends over a longer period than is covered by active nuclear division, so that fully developed plastids are found only occasionally in a cell with a dividing nucleus. Plastids rarely divide until they are fully differentiated and definite in shape and staining qualities. Consequently plastid divisions are most abundant during the latest stages of nuclear division or after it has entirely ceased. (Pls. 3 and 4.)

The independence of plastids and nucleus may be further demonstrated by the indifference of the plastids to nuclear division with no migration of plastids or assembling about the nucleus. The plastids are distributed to the daughter cells by chance, as they happen to lie at one or the other end of the cell at the time of division, and equivalent numbers in the daughter cells are due to the distribution of plastids throughout the mother cell. (Pl. 8, C.) Zirkle (*30*) shows a concentration of the plastid primordia about the nucleus, but Ma (*16*), Kirby (*9*), and Randolph (*21*) failed to find any definite relation of plastid position to the dividing nucleus.

RELATION OF PLASTID NUMBER TO CELL GROWTH

At the time of plastid differentiation the cell is quite compactly filled with the chloroplasts; the cell grows rapidly in size, but the

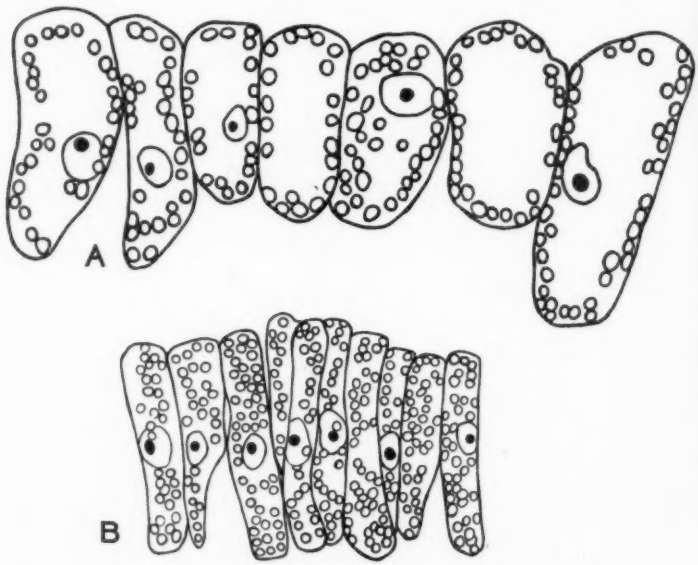
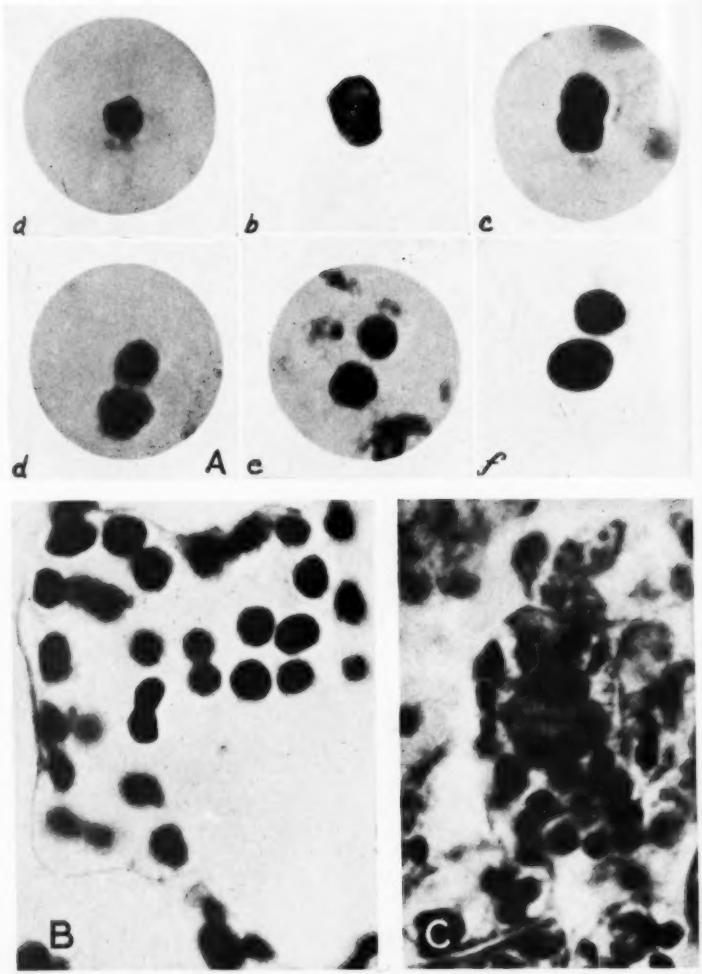


FIGURE 3.—Groups of palisade cells, old (A) and young (B). × 1,100

plastids increase in size and number very slowly. An average of many measurements showed a cell size increase of 300 per cent to be accompanied by a 25 per cent increase in the size of the individual plastids. (Pl. 2, C.) But plastid divisions may cause a numerical increase of 45 per cent. The result of the inequality of the increase of volume of cells and plastids within the cell is shown by the small amount of the space within the older cell taken up by the plastids or other stainable material, as contrasted with that of well-filled young cells. In sections of cells from old leaves the chloroplasts are distributed for the most part only along the cell walls. (Fig. 3.) In a surface section the chloroplasts form single compact layers tightly appressed to the cell wall, the large cell lumen being occupied by the central vacuole. (Pl. 7.)

Surface section of mature palisade cells showing plastids lining the wall, leaving the central lumen free except for nuclei, 8 p. m. fixation, × 2,400



A, Plastids which had become isolated from cells in sections. Successive steps in the elongation and division of an individual plastid to form two new plastids. Note starch in several plastids, and in *f* what is evidently the beginning of a second division. *a–e*, 11 p. m.; *f*, 4.30 a. m. fixation. × 2,400. B, Young cell from spongy parenchyma. Plastids, mature, so distributed that each daughter cell will receive an approximately equal quantity, 11 p. m. fixation. × 2,400. C, Region of spongy tissue showing variety of stages in division among neighboring plastids, even within the same cell, 10 p. m. fixation. × 2,400

TABLE 1.—*Measurements of drawings of 11 blocks of 8 cells each taken from leaves of various sizes and ages to compute number of plastids*

Age of leaves	Area of block of 8 cells in square millimeters	Number of plastids	Plastids per 100 square millimeters
Very young	3,600	192	5
	4,125	270	7
	7,700	329	4
	7,728	368	5
Older	8,320	292	3.5
	8,980	320	3.5
	9,450	221	2
	10,200	392	4
	13,760	408	3
	14,400	496	3.5
Mature	31,140	544	1.7

In Table 1 are given measurements of drawings of 11 blocks of eight cells each taken from leaves of various sizes, ranging from those in which the plastids were barely differentiated to fully mature leaves. It can readily be seen that increase in plastid number does not keep pace with cell growth, for an increase of 765 per cent in cell area was accompanied by an increase of only about 185 per cent in plastid numbers.

TABLE 2.—*Percentage increase in number of plastids as compared with percentage increase in cell size of young and of mature leaves*

Kind of leaves	Increase in cell size	Increase in plastid number
Young	40	14
	90	20
	160	24
Mature	300	45

Table 2 (from another set of measurements) shows a consistent increase in plastid number corresponding, but not equal, to the increase in cell size. A large number of cells of various sizes were measured and the contained plastids were counted. Cells were then grouped together according to size. Using the group of smallest cells as a basis, the percentage of increase in cell size and plastid number was computed for each group.

The proportion of plastid volume to cell capacity does not actually reach such wide variance as the tables seem to indicate, since the plastids grow. In the youngest cells in which plastids were of definite, regular outline the average diameter of 30 plastids was 5.05μ. In the oldest leaves of this material the average diameter of 30 plastids was 6.43μ; an increase of 27 per cent in the size of the plastids. Plastids of fully mature leaves from material which had been fixed from field-grown plants showed an average plastid diameter of 10.38μ or about 100 per cent greater than that of plastids in young leaves. (Fig. 4.) No comparison of cell areas in this material and the young leaf cells could be made, outlines of the old cell walls being too broken and vague.

PROCESS OF DIVISION

Plastid division in the potato leaf follows closely that described for other plants by early workers such as Kny (*10*), Sachs (*24*), and Nageli (*18a*). The gradual growth in the size of the plastid and the change of shape from round to oval (fig. 5, A and B) continues, especially in the longer axis, until the plastid is about twice its original length. A constriction midway between the ends of the elongated plastid followed by a gradual contraction or pinching in of the plastid at this point results in the formation of two new plastids approximately the same size as the original. (Fig. 5, C, D, E, F.) Dividing plastids may be homogeneous throughout or may contain one or more starch grains. (Pls. 8, A, and 9, C.)

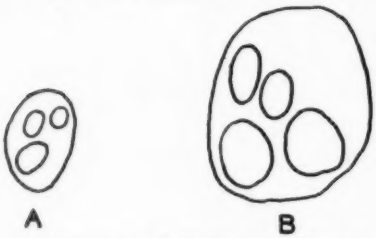


FIGURE 4.—Average plastid from young (A) and old (B) leaves. × 4,200

In some cases dividing chloroplasts may show a clear zone in the region of the constriction (fig. 5, E), the contents becoming concentrated in the two halves. The material in each half rounds off, leaving a membrane between which connects the two new plastids for a time. This bridge may be nearly as wide as the diameter of the plastid or it may be a scarcely visible thread. (Fig. 5, D, E.) This thin

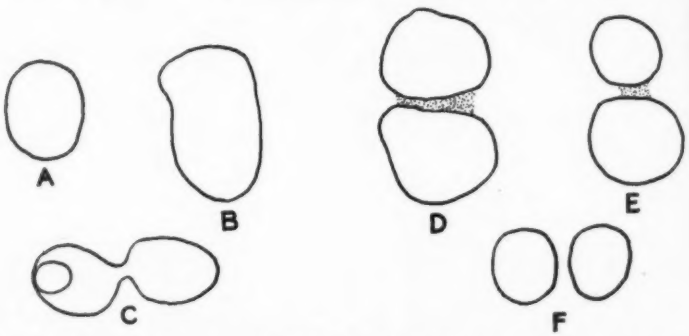
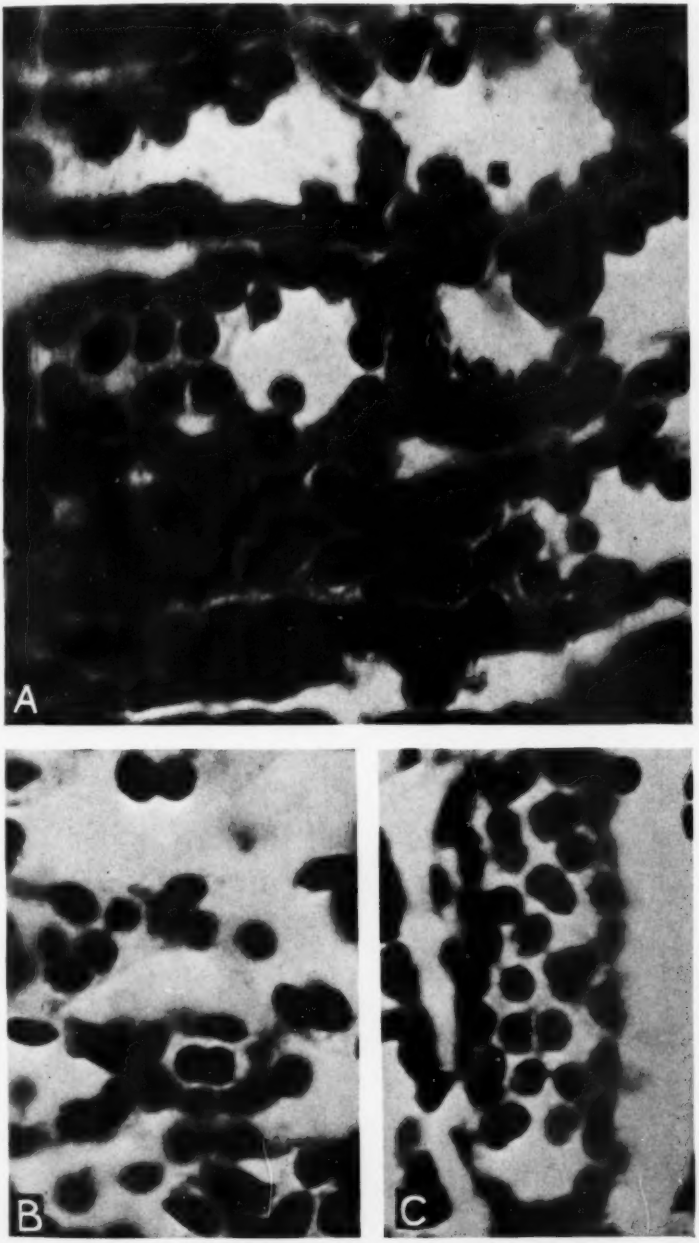


FIGURE 5.—A, Resting plastid; B, constriction of elongated plastid; C–E, various types of connections; F, joining not visible but clearly plastids resulting from division. × 4,200

connection finally disappears and two independent chloroplasts result. Even though no longer joined, the new plastids remain in the same position with reference to each other, so that often in one field the plastids occur in distinct pairs. (Pls. 2, B, 9 and 10.)

Photomicrographs of stained material of division stages in plastids isolated from cells (pl. 8, A) show, with one exception, all chloroplasts with visible starch grains. Elongation and cutting in two of a chloroplast (pl. 8 A, *b*, *c*, *d*) are followed by two newly formed plastids no longer visibly connected but in the same position in which division occurred. (Pl. 8, A, *e*.) The position of members of some groups of



A, Palisade cells showing various plastid divisions within the same or neighboring cells, 6 a. m. fixation. × 2,400. B, A section of spongy parenchyma showing many stages of plastid division, 5.30 p. m. fixation. × 2,400. C, Palisade cells showing several stages of division in plastids; note starch in plastids, 11 p. m. fixation. × 2,400

Palisade cell showing divisions in direction of long axis of cell and newly formed plastids arranged along cell wall. Many other stages in outlying regions. Note rosettelike pattern at *a*, 2 a. m. fixation. × 2,400

plastids suggests the division of those resulting from a former division. (Pl. 8, A, *f*.) Heitz (*6*) found plastid division repeated so rapidly in severed moss leaves that the new plastids divided again in a plane at right angles to the first division before the first division was complete.

RELATION OF STARCH TO DIVISION

It was found that any starch grains present in a dividing plastid are apportioned to the new plastids as they happen to lie with respect to the line of division, thus confirming the findings of Sachs (*24*). (Fig. 6.) The amount of starch contained may vary from none to 1 to 3 large grains or 6 to 8 small ones. The starch is usually evenly distributed so that after division the newly formed plastids receive relatively equal amounts. Starch grains do not appear to hinder division. (Pls. 3, D; 8, A; and 9, C.)

DIURNAL PERIODICITY IN PLASTID DIVISION

Lange (*12*) found a daily periodicity in nuclear divisions, but a tabulation of the hours of day and night at which the material for the present study was fixed shows no such situation regarding plastid division. No attempt was made to count the plastid divisions in day and night material, but an examination of the photomicrographs shows that division is not confined to any particular hour but is going on at all times.

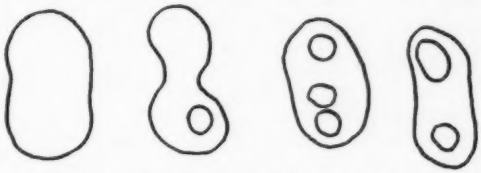


FIGURE 6.—Starch grains in plastids undergoing division. × 4,200

Lower temperatures and relatively high humidity were associated with the hours of darkness but the initiation and rate of plastid division do not seem to be correlated directly with any external factors, such as light, temperature, or humidity.

DIVISION IN CHLOROPLASTS

PHASES OF DIVISION

Increase in plastid number results from the continuous division of the plastids originally differentiated in the cell. No period was determined for the maximum activity in division. Divisions have been found in the plastids which were scarcely fully differentiated (pl. 3, D); but were found in greater abundance in cells of leaves somewhat older. Often all the plastids in one cell were in some stage of division or were arranged in a distinct pattern of pairs as though they had just completed division. (Pl. 2, B.) In any one region all stages appear simultaneously (pls. 9 and 10); and even neighboring plastids may be in very different stages of division (pl. 10).

In young palisade cells with walls compactly lined with plastids divisions occur in a direction diagonal to the wall, so that one of the plastids of the newly formed pair is pushed into the central vacuole. (Pl. 2, B.) In old palisade cells, on the other hand, with walls sparsely lined with plastids, divisions occur parallel to the long axis of the cell

and newly formed pairs lie along the cell wall. (Pl. 10.) Several newly formed plastids may form a rosettelike pattern in which the inner faces of the plastids are angular. (Pl. 10, *a*.)

DISCUSSION

The phenomenon of plastid division has been observed in many plants. Sachs (*24*) suggested that plastid division followed elongation of the plastid and was occasioned by a stretching of the adjacent cell wall. Divisions occur, however, in plastids remote from walls and in planes which do not parallel that of the stretching of the cell wall. Heitz (*6, 7, 8*) claimed that plastid division was a result of the accumulation of products of assimilation. If this were the only factor involved divisions would be expected to occur most frequently when the plastids were filled with these products, i. e., during the day. Although plastid divisions were abundant in material fixed at all hours of the day and night, they were perhaps more plentiful in the material fixed during the night hours; in other words, when the plastids were losing the material temporarily stored during the hours of sunlight. The initiation of plastid division does not seem to be regulated either by the growth of the cell wall or by the accumulated food products. It appears rather to be one of the normal processes which taken together constitute cell growth.

Observations, measurements of cells, and plastid counts indicate that the number of plastids per cell is fairly constant. This being the case, plastid division is not independent of cell development but is merely one phase of the whole process. Cells of certain size contain a characteristic amount of chloroplast material divided into a fairly constant number of bodies which increases as the cell becomes larger.

SUMMARY AND CONCLUSIONS

An investigation of the origin of the chloroplasts in the potato (*Solanum tuberosum*) was made from the point of view of the three well-known theories of plastid origin in other plants.

Attempts to grow plastids from primordia outside the plant in expressed filtered leaf juice failed, either because plastids do not develop from primordia minute enough to pass through a Jenkins filter, because of the toxicity of the potato juice, or because the normal growing conditions in the cell were absent.

Tubers, greened by exposure to light, did not furnish satisfactory material for the study of the origin of the chloroplast. Greening is merely the result of the formation of chlorophyll in undifferentiated regions of the protoplasm. Although, after some time, these patches may assume a definite shape they can not be considered as typical chloroplasts.

A search for chondriosomes or plastid primordia in embryonic leaves by the use of recommended fixing fluids and stains revealed no bodies of this type. In embryonic leaves of the potato, plastids originate from the cytoplasm directly. By gradual vacuolation the cytoplasm is separated into dense masses which round off, become compact, and develop into mature, independent chloroplasts.

Differentiation of chloroplasts from homogeneous cytoplasm does not occur simultaneously throughout all portions of the leaf. Isolated

regions first appear in the spongy tissue, later in the palisade tissue. Differentiation is first completed in the spongy tissue. Plastids in the various regions of a single cell may be in quite different stages of differentiation.

Plastid differentiation seems to be only one phase of the series of changes which together bring about the maturing of a cell. Division of the nucleus occurs at the same time as plastid differentiation but independently. New cell formation usually reaches its maximum before plastid differentation is complete. Plastids, even if formed, show no change of position during nuclear division.

Plastids increase in number as the cell grows, but the increase is much slower than the rate of cell growth. Increase in plastid size compensates somewhat for slow increase in plastid number.

Increase in plastid number is brought about by the division of the original chloroplasts.

Division of chloroplasts is a process of elongation and pinching. The connections between the new plastids may persist for some time. The arrangement of plastids in pairs is very striking even after all visible connections have disappeared. Plastid division figures, if present at all, are found in abundance throughout any one region of the leaf. In general all dividing plastids of a certain region are in approximately the same stage of division, although all stages may occur within a very limited area.

Plastid division seems to be a phenomenon connected with the growth and maturation of the cell.

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SOME NEMIC PARASITES AND ASSOCIATES OF THE MOUNTAIN PINE BEETLE (DENDROCTONUS MONTICOLAE)[1]

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INTRODUCTION

A study of the nemic parasites and associates of bark beetles is of both economic and scientific interest. Some of the new species proposed by Fuchs [3] [4] in his recent works on the nemic fauna of the bark beetles of central Europe must be considered detrimental to the beetles and therefore of economic importance. The limited observations recorded in the present paper indicate that the nemic fauna of all bark beetles known to occur in the United States should be investigated, as some of the nemas may prove of value in the control of the beetles by acting as disease carriers and as consumers of weak or even healthy beetles and their eggs.

Closely related to the determination of economic importance is the study of the ecological relations existing between the nemas and the beetles. Of special interest also is the classification of the various genera and species. Both of these latter aspects of the subject are discussed in the present paper.

MATERIAL

The material used in the present investigation was collected at Metaline Falls, Wash., in July and August, 1930. Some of it was taken from under the bark of a pine that had been killed by the mountain pine beetle (*Dendroctonus monticolae* Hopk.) in 1929 and some from a pine that had become infested not more than 10 days before the date of collection, July 18, 1930.

ECOLOGY

RELATION OF NEMAS TO BARK BEETLES

Some of the new nemic genera proposed by Fuchs [5] are extremely close to free-living genera, and the various species represent almost every intermediate stage, thus illustrating the manner in which parasitic nemas may arise from free-living forms. From a mere occurrence of the nemas in the mines of the bark beetles and their possibly accidental use of the beetles as carriers, the way to true parasitism seems to proceed by the following steps: (1) The use of the bark beetle as unconditional carrier, (2) the extension of the carrier association into a regular life association under the wing covers or on

[1] Received for publication Jan. 21, 1932; issued October, 1932.

[2] The material for this investigation was collected by D. DeLeon, formerly of the forest insect field station, Bureau of Entomology, U. S. Department of Agriculture, at Coeur D'Alene, Idaho.

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[5] FUCHS, G. Op. cit. (See footnote 3.)

the exterior surface of the beetle, (3) the production of special cocoons and webs by the nemas on the body of the beetle, (4) the habit of feeding on the beetle itself from the outside (ectoparasitism), and (5) true endoparasitism of the nemic larvae.

ECOLOGICAL GROUPS OF APHELENCHOIDES

Certain species of Aphelenchoides [6] are free-living; others are parasitic, either on plants or animals; and there are numerous intermediate species. Fuchs separates his Parasitaphelenchus into the three following ecological groups:

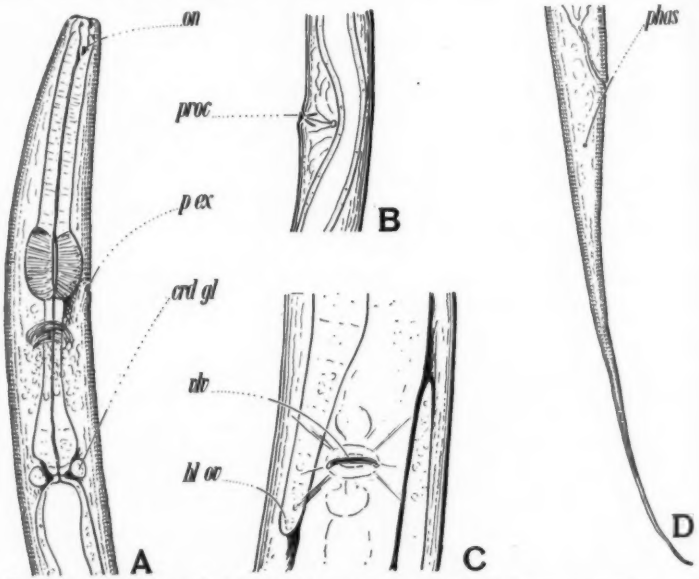

FIGURE 1.—*Diplogaster occidentalis* (female). A.—Head end in lateral view: *on*, onchium; *p ex*, excretory pore; *crd gl*, cardiac gland. × about 750. B.—Vulvar region in lateral view: *proc*, toothlike process. × about 530. C.—Vulvar region in ventral view: *vlv*, vulva; *bl ov*, blind end of anterior ovary. × about 1,090. D.—Tail: *phas*, phasmid. × about 530

(1) Species whose larvae live as parasites in the body cavity of bark beetles. (Endoparasites.)

(2) Species whose larvae are found with or without a cocoon either under the wing covers of bark beetles or attached sucking on the backs of the beetles. (Ectoparasites.)

(3) Species whose larvae and adults are found in the frass of the mines or on the beetles themselves, but under conditions not indicative of true parasitism. (Free-living.)

This classification outlines the three main aspects of the relationship between the species of Aphelenchoides and the bark beetles. The two species described in a later section of the present paper, from the mountain pine beetle of Washington, apparently represent true endoparasites. Only adults were seen, but it is highly probable that the larvae develop within the body cavity of the beetles. A still more pronounced type of parasitism is possible—that in which not only the larvae but also the adults are endoparasites. That this type of

[6] Synonyms: Pathoaphelenchus Cobb, and Parasitaphelenchus Fuchs. (See footnotes 4 and 10.)

parasitism occurs in the genus Aphelenchoides is not known, but it may be considered possible in view of the present meager knowledge of these forms.

A number of other genera described by Fuchs [7] from various bark beetles show a trend toward parasitic life, but the process is especially impressive in Aphelenchoides because the most closely related forms, such as species of Aphelenchus, Paraphelenchus, and Schistonchus, are well-known free-living or plant-parasitic species. A somewhat new conception of the status of these genera is suggested in the present paper.

DIPLOGASTER AND THE MOUNTAIN PINE BEETLE

Tendencies toward parasitism are evident in the genus Diplogaster, although at present there is no species known that may properly be called a parasite. The species described herein is considered by the writer to be an associate, rather than a parasite, of the mountain pine beetle. These nemas inhabit the mines and frass and are probably carried by the beetles from one mine, tree, or locality to another.

TAXONOMY

DIPLOGASTER OCCIDENTALIS, N. SP.

Diplogaster occidentalis (figs. 1–3) is closely related to a group of forms, namely, *D. rhodani* Stefanski, *D. nudicapitatus* Steiner, *D. lineatus* Fuchs, and *D. consobrinus* De Man.[8] Of these four forms, only *D. lineatus* is described from bark beetles. It was found in the mines of *Hylobius abietis* L. from Europe. *D. occidentalis* is also found living in bark-beetle mines. In all, 175 specimens were observed, including 47 males, 92 females, and 36 larvae.

FIGURE 2.—*Diplogaster occidentalis*. A.—Front view of head: *ppl*, papilla; *amph*, amphid; *on*, onchium. × about 1,610. B.—Free-hand sketch of pharynx

The largest males are about one and one-half times as large as the smallest. Males, females, and larval specimens have an elongated filiform tail; in the male the base is somewhat set off from the slender end portion. (Fig. 3, C.) The cuticle has 18 equidistant uninterrupted longitudinal wings of the same height. (Fig. 3, B.) The intermediate portions of the cuticle, however, show annulation. A cross section shows the lateral and ventral cords to be rather wide (fig. 3, B); no dorsal cord was seen. The head structures are somewhat obscure. There seem to be six lips, each having a papilla. (Fig. 2, A.) In a front view, small slits just behind the lateral papillae are interpreted as being the external amphids. The oral opening is triangular in shape, with the three corners well cuticularized and distinct. The form of the pharynx is rather indistinct; its walls are not well cuticularized and are only partly thickened (fig. 2, B); it is narrow but deep, and of somewhat irregular shape. What appears to be an onchium is situated at its base; for an onchium, however, it is little cuticularized. The anterior cylindrical portion of the esophagus is followed by a well-developed medial bulb, which exhibits numerous radial muscle fibers and long but thin valvulae. The isthmus and terminal bulb are typically diplogastroid, clear and rather transparent. Three cardiac glands were seen. The excretory pore opens ventrad of the middle bulb. The female apparatus is amphidelphic, with the reflexed ovaries extending back to the vulva or even past it. (Fig. 1, C.) The transparent vulva is slitlike (fig. 1, C); its posterior lip, in profile view, seems to form a toothlike process (fig. 1, B). The male has a single reflexed testis. (Fig. 3, A.)

[7] FUCHS, G. Op. cit. (See footnote 4.)

[8] MAN, J. G. DE. DESCRIPTION D'UNE ESPÈCE DU GENRE DIPLOGASTER MAX SCHULTZE: DIPLOGASTER CONSOBRINUS NOV. SP. Ann. Soc. Roy. Zool. Malacol. Belg. 51: 47–54, illus. 1920.

Its copulatory apparatus is remarkable because of the large-sized gubernaculum—nearly as long as the spicula—especially well seen in side view. Linear distally, it widens considerably toward its inner end, which forms a rather wide plate ending with straight transverse truncation. The spicula are much finer; their proximal ends distinctly capitate. Distally the gubernaculum exhibits in side view a larger and, proximad to it, a smaller process; the ventral view, however, shows only the larger process, which then appears very prominent. (Fig. 3, C.) The protrusile muscle of the gubernaculum is remarkably strong; oblique copulatory muscles occur in front of the anus. The arrangement of the male copulatory papillae is shown in Figure 3, C and D. Of special interest are the

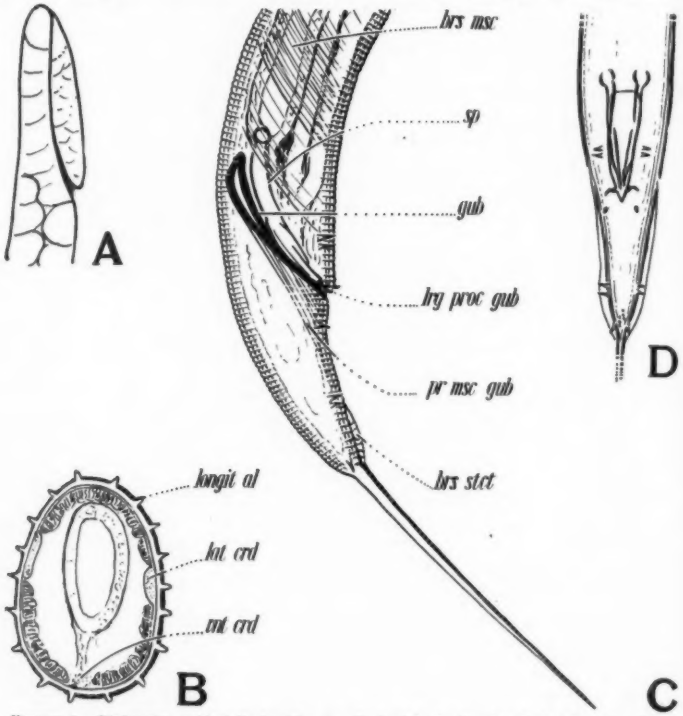

FIGURE 3.—*Diplogaster occidentalis* (male). A.—End of testis (free-hand sketch). B.—Cross section in middle region of body anterior to testis: *longit al*, longitudinal wing; *lat crd*, lateral cord; *vnt crd*, ventral cord. × about 1,070. C.—Tail end: *brs msc*, bursal muscles; *sp*, spiculum; *gub*, gubernaculum; *lrg proc gub*, larger process of the gubernaculum; *pr msc gub*, protrusile muscle of the gubernaculum; *brs stct*, bursalike structure. × about 1,070. D.—Anal region in ventral view. × about 665

small winglike or bursalike formations on each side of the tail of the male. (Fig. 3, D.) One of the papillalike lateral structures apparently connected with this wing may be a phasmid, which is clearly seen on the female tail. (Fig. 1, D.)

MEASUREMENTS:

Male:	1. 1	13. 0	19. 0	'M	81. 0	0. 60 mm.
	1. 9	4. 5	4. 8	5. 6	3. 7	
Male:	1. 1	13. 0	19. 0	'M	80. 0	0. 43 mm.
	1. 5	3. 9	3. 9	3. 9	3. 2	
Female:	0. 7	13. 0	17. 0	'48.'	76. 0	0. 64 mm.
	1. 3	3. 0	3. 5	4. 1	2. 8	
Female:	0. 72	14. 0	18. 0	'46.'	75. 0	0. 625 mm.
	1. 40	3. 6	3. 6	4. 2	2. 2	

DIAGNOSIS.—Diplogaster with 18 equidistant longitudinal wings, with distinct annulation in the interspaces. Head with six lips, each of which has a single papilla. Pharynx narrow, irregular, much longer than wide, without distinct separation into wider anterior and a narrow posterior portion, and with an indistinct, little cuticularized dorsal onchium at its base. Middle esophageal bulb with long but thin valvulae. Female apparatus amphidelphic, ovaries reflexed, ending past the vulva; posterior vulvar lip in profile view with small toothlike structure. Male and female tail long, conical at base, filiform at end portion. Male with a very prominent gubernaculum, at least five-sixths of the length of the spicula; copulatory papillae as shown in Figure 3, C and D.

THE GENUS APHELENCHOIDES

STATUS OF THE GENUS

Two species from the material used in the present study were found to be very closely related to a number of species recently described by Fuchs [9] under the generic name of Parasitaphelenchus. Unfortunately Fuchs failed to give a generic diagnosis. His description reveals no characters that apply to all his species or differentiate them from other genera such as Aphelenchoides. All his species and also the two new species described in the present paper may properly be placed in the latter genus. The generic name Aphelenchoides is here used in the sense suggested by Cobb for Pathoaphelenchus [10] (which is antedated by the name Aphelenchoides [11]). Its characters, which are here repeated, are those of the former genus Aphelenchus, but not those that must be recognized since the discovery of the males of the type species of Aphelenchus, *A. avenae*. This species exhibits characters that separate it widely from the forms with which it has hitherto been associated and which must now be included in the genus Aphelenchoides. The writer [12] [13] has given a fuller discussion of this question elsewhere.

Fuchs [14] mentions for four of his Parasitaphelenchus species a character which, if proved to be valid, would fully justify the creation of a new genus, namely, the location of the nerve ring in front of the esophageal bulb in the species *Parasitaphelenchus typographi*, *P. dubius*, *P. conjunctus*, and *P. hylastophilus*. This position of the nerve ring, if established, is exceedingly remarkable, so much so that further observations must be made before it can be accepted. If, however, it is accepted as a generic character, most of the species included by Fuchs in the genus Parasitaphelenchus would have to be excluded.

At present, therefore, it is not possible to recognize Parasitaphelenchus as a genus in good standing, but it should be synonymized with Aphelenchoides. If a division of the latter genus is to be made, it could only be on characters that are not yet entirely clear and certain. If the future should show the need for the creation of a new genus, its characters will have to be formulated anew.

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[9] FUCHS, G. Op. cit. (See footnote 4.)

[10] COBB, N. A. [APHELENCHUS RETUSUS WITH A PROPOSED DIVISION OF APHELENCHUS.] Helminthol. Soc. Wash. Proc., Jour. Parasitol. 14: 57. 1927.

[11] FISCHER, M. ÜBER EINE CLEMATIS-KRANKHEIT. Ber. Physiol. Lab. Landw. Inst. Halle 3 (Heft 11): 1-11, illus. 1894.

[12] STEINER, G. ON THE STATUS OF THE NEMIC GENERA APHELENCHUS BASTIAN, PATHOAPHELENCHUS COBB, PARAPHELENCHUS MICOLETZKY, PARASITAPHELENCHUS FUCHS, ISONCHUS COBB AND SEINURA FUCHS. Jour. Wash. Acad. Sci. 21: 468-475, illus. 1931.

[13] ——— ANNOTATIONS ON THE NOMENCLATURE OF SOME PLANT PARASITIC NEMATODES. Jour. Wash. Acad. Sci. (In press.)

[14] FUCHS, G. Op. cit. (See footnote 4.)

The generic characters of Aphelenchoides are as follows:

(1) The males have no bursa but only copulatory papillae; the spicula are short, strongly arcuate, proximally wide to very wide, distally more or less pointed; the gubernacula are absent; the testis is single.

(2) The postbulbar portion of the esophagus is more or less distinctly assimilated by the intestine, which therefore appears to begin immediately behind the esophageal bulb. This latter is the homologue of the middle esophageal bulb in Tylenchus and other genera.

(3) The outlets of the three salivary (esophageal) glands are located in the esophageal bulb, the dorsal one anterior and the two ventro-submedial ones posterior to the valvulae.

(4) The females are prodelphic with a more or less reduced posterior gonadic branch still present.

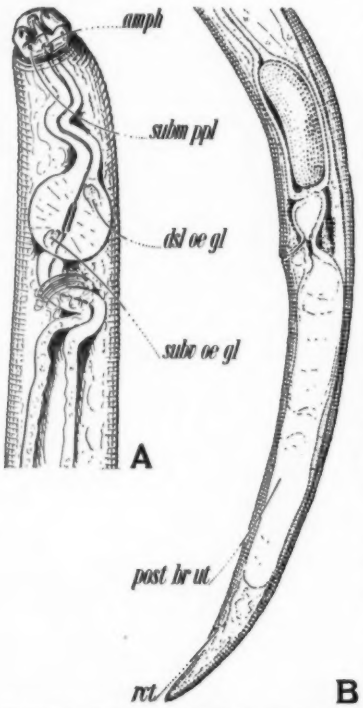

FIGURE 4.—Parts of *Aphelenchoides conurus*. A.—Head end of female: *amph*, amphid; *subm ppl*, submedial papilla; *dsl oe gl*, outlet of dorsal esophageal gland; *subv oe gl*, outlet of subventral esophageal gland. × about 530. B.—Posterior portion of female: *post br ut*, posterior branch of uterus; *rct*, indistinct rectum. × about 360

APHELENCHOIDES CONURUS, N. SP.

Only two specimens of *Aphelenchoides conurus* (fig. 4) were seen, and unfortunately one of them was lost during preparation. The following description is therefore based mainly upon a single female.

The tail is elongate-conical, but, in contrast to other species, not mucronate. The cuticle is annulated but has no wings. The specimen observed had a distinct lip region which exhibited all the characters typical of the genus. There are four submedial papillae, and laterally rather distinct amphidial openings. The spear is short but rather wide; notable is the absence of knots or swellings at its proximal end. The spear thus offers the best specific characters. The esophageal bulb is exceptionally large (fig. 4, A); its valvulae are distinct but not prominent; radial muscle fibers were not seen. The ampullae and outlets of the salivary or esophageal glands could be distinguished; they seem to have the normal position, that is, the position typical for Aphelenchoides. The dorsal gland opens in the anterior portion of the bulb and the two subventral ones in the posterior. The intestine begins at the bulb as a cylindrical tube which, however, swells after it has passed through the nerve ring. Its lumen is very indistinct; its tissue is filled with reserve material, the cell walls being therefore obscure. The rectum, too, is rather indistinct, perhaps not even functional. The excretory pore was not seen. The female apparatus is prodelphic, but, as in other members of the genus, a large posterior branch of the uterus extends nearly to the anus. The vagina leads slightly forward. The eggs are about three times as long as wide.

MEASUREMENTS:

Female:	0. 8	6. 3	7. 0	80. 0	96. 3	1.0 mm.
	1. 1	2. 3	2. 4	2. 8	1. 3	

DIAGNOSIS—Aphelenchoides with elongated, conical nonmucronate tail. Spear short but wide, without basal swellings. Esophageal bulb large, spherical, apparently without radial fibers. Living as parasite and associate of the mountain pine bettle.

APHELENCHOIDES ACROPOSTHION, N. SP.

Several females and males of *Aphelenchoides acroposthion* were found. Compared with other members of the genus, this species is a very large form.

Although the specimens had the appearance of true parasites, no exact facts were known as to their relationship to the mountain pine beetle except that they have been found in the mines of the beetles. Since only adult specimens were present, one is led to suppose that their larvae may be endoparasites of the beetle.

The body is very slender and elongate. The tail of the female is short and bluntly rounded (fig. 5, A); that of the male is also short, and its base is similar to that of the female but with the difference that it ends in a short hornlike process (fig. 5, D). The cuticle is finely annulated, but no lateral membrane was seen. The distinctly set-off lip region exhibits in a front view six rounded, well-separated lips. (Fig. 5, B.) A cuticularized ring with six short rays encircles the oral opening. The structures of the head end were rather obscure, but it is believed that the amphids were seen in the position shown in Figure 5, B. This species, like other members of the genus, has four submedial papillae. The spear is extremely fine (fig. 5, E) and appears to be composed of three different portions—an anterior conical portion rather well cuticularized, a succeeding short cylindrical portion also well cuticularized, and then a long cylindrical portion which is hardly cuticularized and which, posteriorly, is set off from the esophageal canal only by the attachment of the protruding spear muscles. No basal swellings were seen. The esophageal bulb is well developed and of oval shape; it has rather long but thin valvulae. (Fig. 5, F.) The radial muscles attached to the valvulae exclude a more glandular portion at the anterior and posterior ends of the bulb. The connection of the intestine with this bulb is much the same as in other forms of Aphelenchoides. The nerve ring occurs a short distance behind the bulb. The intestine is of somewhat degenerate character; its cell walls can hardly be recognized, the whole organ being filled with reserve material. As in the previous species, the rectum and anal opening are extremely fine and obscure. No excretory pore was seen. The vulvar opening is well marked because the body narrows just behind it. The female sexual apparatus is prodelphic; there is, however, a well-developed posterior branch of the uterus, and attached to it there appears to be a vestigial ovary extending nearly to the anus. The anterior ovary extends forward nearly to the nerve ring. The very short sexual apparatus of the male is of the proorchid type, with the end of the testis reflexed. Figure 5, D, gives a lateral view, and Figure 5, C, a ventral view of the copulatory apparatus. It seems that the two spicula are amalgamated, forming a single spiculum, pointed at the outer end, but very wide at the inner end. The ventral apophysis exhibits at its inner end a short process pointing ventrad; the dorsal apophysis is somewhat swollen proximally. Only one male was studied in ventral view; it showed in the anal region a peculiar lateral expansion somewhat resembling a vestigial bursa. Two papillae were distinctly seen on the inside of this expansion, one in the anal region and the other at the base of the hornlike process. It is doubtful whether these males are fully functional.

MEASUREMENTS:

Male:	0. 8	5. 7	6. 2	M	99. 2	1.6 mm.
		0. 9	0. 9	1. 6	0. 6	
Female:	0. 5	3. 5	4. 1	$^{78}82.3^{16}$	92. 0	99. 0	2.4 mm.
	0. 4	0. 8	0. 8		1. 2	0. 9	

DIAGNOSIS.—Aphelenchoides of long, slender shape. Tail of female short, obtusely rounded; that of male broadly conical at the base, mucronate. Spear rather narrow, of average length, without basal swellings. Esophageal bulb ovoid. Vulva well marked because the body narrows abruptly behind it. Male with slightly sublateral papilla in latitude of the anus, and another somewhat in front of terminal process.

SUMMARY

Some nemic associates and parasites of the mountain pine beetle are described and their economic significance is discussed. The various phases of the mutual relationship of the bark beetle and the nemas are considered (free association, carrier relationship, ectoparasites, endoparasites). The taxonomy of the genus Aphelenchoides and three nemas new to science, *Diplogaster occidentalis*, *Aphelenchoides conurus*, and *A. acroposthion*, are discussed and morphological descriptions given.

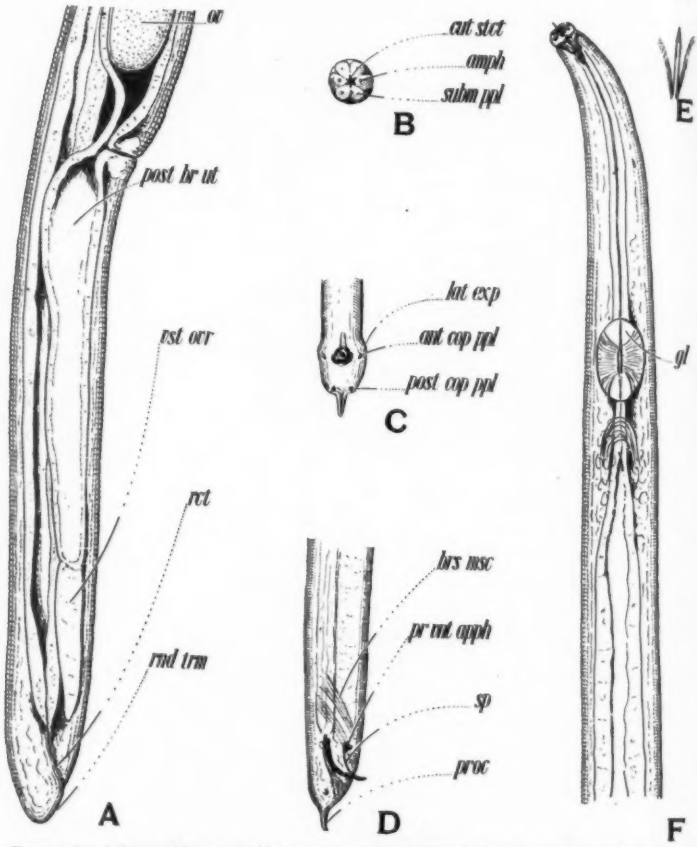

FIGURE 5.—*Aphelenchoides acroposthion*. A.—Posterior portion of female: *ov*, egg; *post br ut*, posterior branch of uterus; *vst ovr*, vestigial ovary; *rct*, rectum; *rnd trm*, rounded tail. × about 720. B.—Front view of head: *cut stct*, cuticularized structure; *amph*, amphid; *subm ppl*, submedial papilla. × about 940. C.—Tail of male, ventral view: *lat exp*, lateral expansion; *ant cop ppl*, anterior copulatory papilla; *post cop ppl*, posterior copulatory papilla. × about 720. D.—Tail of male, lateral view: *brs msc*, bursal muscles; *pr vnt apph*, process of ventral apophysis; *sp*, spiculum; *proc*, hornlike process (mucro.). × about 720. E.—Free-hand sketch of spear. F.—Anterior portion of body: *gl*, granulated portion of esophageal bulb. × about 720

THE VITAMIN A, B, C, AND G CONTENT OF CONCORD GRAPES[1]

By ESTHER PETERSON DANIEL, *Associate Nutrition Chemist*, and HAZEL E. MUNSELL, *Senior Nutrition Chemist in Charge, Nutrition Studies Section, Foods and Nutrition Division, Bureau of Home Economics, United States Department of Agriculture*

INTRODUCTION

During an earlier study [2] the vitamin A, B, C, and G content of Sultanina (Thompson Seedless) and Malaga grapes as well as two brands of commercial grape juice was determined. Since the completion of this work, there have been numerous requests for information concerning the vitamin content of Concord grapes (*Vitis labrusca*), and in consequence the study presented here was conducted.

PLAN OF STUDY

The Concord grapes tested were purchased at retail stores and were therefore representative of the fruit available to the consumer. Only the edible portion of the fruit was used in the experiments. This portion consisted of as much pulp and juice as could be removed from the skins, which were discarded as nonedible. The edible portion of the fruit was fed in 2, 4, and 6 g daily amounts in the tests for the vitamin A, B, and G content, and in 10, 12, and 15 g daily allotments in the vitamin C determination. Eight rats were fed each dose tested for vitamin A, B, and G, and seven guinea pigs were used in the vitamin C determinations.

Following the technic used in the earlier studies, vitamin A was determined by the Sherman and Munsell method,[3] vitamin B by a method worked out in this laboratory similar to one outlined by Chase,[4] vitamin C by the histological procedure described by Höjer,[5] and vitamin G by the writers' own method, which is not unlike that used by Sandels.[6] The diets and procedures used in determining each of these vitamins are given in the earlier paper.[7]

RESULTS

A summary of the data obtained in the vitamin A determination, illustrated in Figure 1, shows that 6 g of the edible portion of Concord grapes fed as the sole source of vitamin A induced but very little growth in the rats during the 8-week test period. From this evidence

[1] Received for publication June 15, 1932; issued October, 1932.

[2] DANIEL, E. P. and MUNSELL, H. E. THE VITAMIN A, B, C, AND G CONTENT OF SULTANINA (THOMPSON SEEDLESS) AND MALAGA GRAPES AND TWO BRANDS OF COMMERCIAL GRAPE JUICE. Jour. Agr. Research 44: 59–70, illus. 1932.

[3] SHERMAN, H. C., and MUNSELL, H. E. THE QUANTITATIVE DETERMINATION OF VITAMIN A. Jour. Amer. Chem. Soc. 47: 1639–1646, illus. 1925.

[4] CHASE, E. F. A QUANTITATIVE STUDY OF THE DETERMINATION OF THE ANTI-NEURITIC VITAMIN (F OR B1). 41 p., illus. New York, 1928. (Thesis, Ph. D., Columbia Univ.)

[5] HÖJER, J. A. STUDIES IN SCURVY. Acta Paediatrica 3; Supplementum, 278 p., illus. Uppsala. 1924.

——— METHOD FOR DETERMINING THE ANTISCORBUTIC VALUE OF A FOODSTUFF BY MEANS OF HISTOLOGICAL EXAMINATION OF THE TEETH OF YOUNG GUINEA-PIGS. Brit. Jour. Expt. Path. 7: 356–360, illus. 1926.

[6] SANDELS, M. R. RELATIVE SOLUBILITIES OF THE ANTINEURITIC AND ANTIPELLAGRIC VITAMINES IN ALCOHOL, AND A STUDY OF CERTAIN PROPERTIES OF THESE SUBSTANCES. 71 p., illus. New York. 1928. (Thesis, Ph. D., Columbia Univ.)

[7] DANIEL, E. P., and MUNSELL, H. E. Op. cit.

it must be concluded that the pulp and juice portion of the Concord grape is a poor source of vitamin A. The edible portions of the Sultanina (whole) and Malaga (seeded) grapes, which contain only small amounts of vitamin A, as shown by the earlier investigation, are nevertheless a richer source of this vitamin than the edible portion of Concord grapes. This is evident from the fact that 5 g daily of

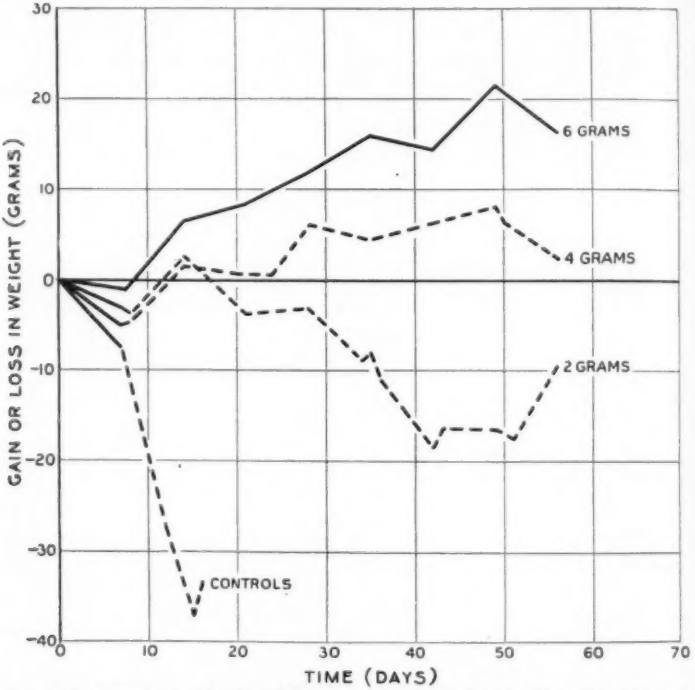

FIGURE 1.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin A. The broken lines begin at a point indicating the occurrence of the first death in the group. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

Sultanina or Malaga grapes produced a greater gain in weight than did 6 g daily of Concord grapes.

The results of the vitamin B tests, portrayed graphically in Figure 2, show that there are very small but detectable amounts of vitamin B in the edible portion of Concord grapes. In contrast to these findings, Malaga (seeded) and Sultanina (whole) grapes proved to be fair sources of this vitamin.

Although 10, 12, and 15 g quantities of the Concord grape pulp and juice were fed daily as a source of vitamin C, the results of a histological examination of the teeth of the experimental guinea pigs showed no evidence of protection. The same quantity of Malaga (seeded) and Sultanina grapes (whole), while insufficient to give complete protection, contained small amounts of vitamin C.

There is no demonstrable amount of vitamin G in the edible portion of Concord grapes. (Fig. 3.) Malaga (seeded) grapes also

showed no evidence of the presence of this vitamin, while Sultanina (whole) grapes contained only a very slight quantity.

The edible portions of the Malaga (seeded) and Sultanina (whole) grapes were, with one exception, richer in each of the vitamins tested

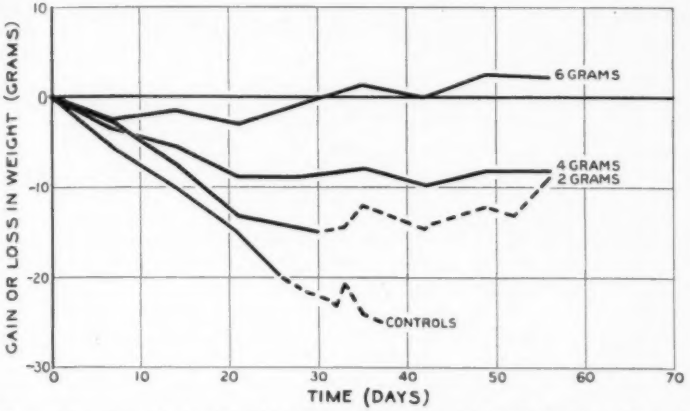

FIGURE 2.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin B (antineuritic). The broken lines begin at a point indicating the occurrence of the first death in the group. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

than was the edible part of the Concord grapes; the Malaga and Concord grapes were both lacking in vitamin G. It may be possible that some of the vitamin value of the Concord grapes was lost when

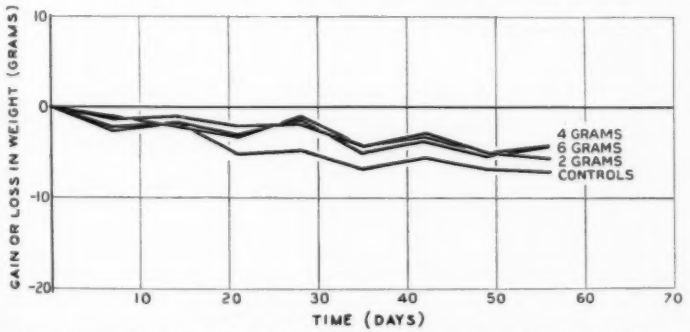

FIGURE 3.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin G. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

the skins were discarded. The skins were retained in the tests made on Malaga and Sultanina grapes. It is known that in the case of some other fruits vitamins tend to be concentrated in the skin or in the portion directly beneath the skin.

SUMMARY

The vitamin A, B, C, and G content of the edible portion of Concord grapes (*Vitis labrusca*) was determined. The edible portion was considered as the part remaining after the removal of the skins.

Results showed that this portion of Concord grapes contains a very small amount of vitamin A and barely detectable amounts of vitamin B. There was no evidence of the presence of either vitamin C or vitamin G.

Earlier studies indicate that edible portions of Malaga (seeded) and Sultanina (whole) grapes (*Vitis vinifera*) possess a greater vitamin A, B, and C content than does the edible portion of Concord grapes. Sultanina grapes appear to contain a small amount of vitamin G, which makes them a richer source of this vitamin. One explanation for these differences may be found in a possibly greater concentration of vitamin in the skins, which were retained in the case of the Sultanina and Malaga grapes and were discarded as a nonedible portion of the Concord grapes.

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